

PATENT APPLICATION

5 **SOLUBLE, STABLE FORM OF HDM2, CRYSTALLINE
FORMS THEREOF AND METHODS OF USE THEREOF**

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**SOLUBLE, STABLE FORM OF Hdm2, CRYSTALLINE FORMS THEREOF
AND METHODS OF USE THEREOF**

This application claims the benefit of United States Provisional

5 Applications 60/461,787, filed April 10, 2003, and 60/547,265, filed February 24, 2004. These applications are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to a soluble and stable form of human Double Minute 2 protein, Hdm2. The present invention further pertains to nucleic acids encoding these proteins. The present invention also relates to a process of obtaining specific samples of Hdm2 that are amenable to forming homogeneous crystals for X-ray crystallization analysis and the crystals formed thereby. The present invention also pertains to methods of using the X-ray diffractable crystals in structure-based drug design to identify compounds that 15 can modulate the activity of the protein.

BACKGROUND OF THE INVENTION

The most commonly inactivated tumor suppressor gene in human cancer encodes the p53 protein, a transcription factor that is intimately involved in maintaining the integrity of the genome in a cell [Hall and Peters, *Adv. Cancer Res.*, **68**:67-108 (1996); Hainaut *et al.*, *Nucleic Acid Res.*, **25**:151-157 (1997); Sherr, *Cancer Res.*, **60**:3689-95 (2000)]. In response to oncogenic stress signals, the cell triggers the p53 transcription factor to initiate either apoptosis or cell cycle arrest. Apoptosis facilitates the elimination of damaged cells from the organism, while cell cycle arrest enables damaged cells to repair genetic damage [reviewed in Ko *et al.*, *Genes & Devel.* **10**:1054-1072 (1996); Levine, *Cell* **88**:323-331 (1997)]. The loss of the safeguard functions of p53 predisposes damaged cells to progress to a cancerous state. Inactivating p53 in mice 25 consistently leads to an unusually high rate of tumors [Donehower *et al.*, *Nature*, **356**:215-221 (1992)].

The p53 transcription factor promotes the expression of a number of cell cycle regulatory genes, including the gene encoding the Mouse Double Minute (Mdm2) protein [see, Chene, *Nature Reviews Cancer* 3:102-109 (2003)]. The Mdm2 protein (designated Hdm2 in humans and Mdm2 in mice) acts to down-

5 regulate p53 activity in an auto-regulatory manner [Wu *et al.*, *Genes Dev.*, 7:1126-1132 (1993); Barak *et al.*, *EMBO J.*, 12:461-468 (1993)]. In the absence of oncogenic stress signals, *i.e.*, under normal cellular conditions, the Mdm2 protein serves to maintain p53 activity at low levels [Wu *et al.*, *Genes Dev.*, 7:1126-1132 (1993); Barak *et al.*, *EMBO J.*, 12:461-468 (1993)].

10 Interestingly, whereas Mdm2 negative ($Mdm2^{-/-}$) mice are not viable [Jones *et al.*, *Nature*, 378:206-208 (1995); Montes de Oca Luna *et al.*, *Nature*, 378:203-206 (1995)], additional inactivation of the p53 gene rescues $Mdm2^{-/-}$ mice [Jones *et al.*, *Nature*, 378:206-208 (1995); Montes de Oca Luna *et al.*, *Nature*, 378:203-206 (1995)]. These results indicate that the misregulation of the 15 p53 transcription factor in the Mdm2 negative mice is the root cause of the observed lethality of the $Mdm2^{-/-}$ genotype, and that the regulation of p53 function relies on an appropriate balance between the two components of this p53-Mdm2 auto-regulatory system. Indeed, this balance appears to be essential for cell survival.

20 There are at least three ways that Mdm2 acts to downregulate p53 activity. First, Mdm2 can bind to the N-terminal transcriptional activation domain of p53 to block expression of p53-responsive genes [Kussie *et al.*, *Science*, 274:948-953 (1996); Oliner *et al.*, *Nature*, 362:857-860 (1993); Momand *et al.*, *Cell*, 69:1237-1245 (1992)]. Second, Mdm2 shuttles p53 from the nucleus to the 25 cytoplasm to facilitate the proteolytic degradation of p53 [Roth *et al.*, *EMBO J.*, 17:554-564 (1998); Freedman *et al.*, *Mol Cell Biol*, 18:7288-7293 (1998); Tao and Levine, *Proc. Natl. Acad. Sci.* 96:3077-3080 (1999)]. Finally, Mdm2 possesses an intrinsic E3 ligase activity for conjugating ubiquitin to p53 within the ubiquitin-dependent 26S proteosome pathway [Honda *et al.*, *FEBS Lett.*, 30 420:25-27 (1997); Yasuda, *Oncogene* 19:1473-1476 (2000)]. Thus, Mdm2 impedes the ability of the p53 transcription factor to promote the expression of its target genes through binding p53 in the nucleus.

Attenuating the p53-Mdm2 auto-regulatory system can have a critical effect on cell homeostasis. Consistently, a correlation between the overexpression of Mdm2 and tumor formation has been reported [Chene, *Nature* 3:102-109 (2003)]. Since Mdm2 acts as a post-translational regulatory effector of the p53 transcription factor, compounds that hinder the ability of Hdm2/Mdm2, to interact with p53 would be anticipated to cause an immediate increase in p53 activity, and thereby rapidly promote either cell cycle arrest or apoptosis in damaged cells. Not surprisingly then, there is currently a substantial effort being made to identify new anticancer agents that hinder the ability of Hdm2 to interact with p53 [Chene, *Nature* 3:102-109 (2003)]. However, to date, no suitable anticancer agent has been found.

Structure-based drug design is one way to optimize the success of identifying useful antagonists of Hdm2, but use of this powerful methodology requires the three-dimensional structure of the target protein. So far, little information has been provided regarding the three-dimensional structure of Hdm2. Indeed, the only structures of Mdm2 currently available are those of Hdm2 and *Xenopus* Mdm2 (XMdm2), complexed with a p53 peptide, but neither crystalline form is suitable for structure-based drug design [Kussie, *et al. Science*, 274(5289): 948-953 (1996)]. Moreover, most of the protein-protein contacts in these crystal lattices are formed through the interaction of the exposed residues in the bound p53 peptide (D21, K24, and L25), making the p53 peptide difficult to displace, which makes it inaccessible to potential inhibitors.

In direct contrast, a successful structure-based drug design program focusing on Hdm2 requires a form of the Hdm2 protein that is amenable to crystallization in the absence of any particular binding partner. Further, the crystal form of the p53-binding pocket of the Hdm2 protein should be accessible to potential inhibitors used for testing binding or for co-structural determination. However, up until now, the solubility and stability of the free Hdm2 protein has been significantly less than that of the Hdm2-p53 peptide complex.

Thus, there is a need to obtain nucleic acids that encode an Hdm2 protein that is soluble and stable at high protein concentrations even when the protein is free of p53 or fragments thereof. In addition, there is a need to design

purification procedures that lead to the preparation of an isolated active Hdm2 protein that is soluble and stable when independent of p53 or fragments thereof. In addition, there is a need to obtain reproducible crystals of Hdm2 that are of sufficient quality for X-ray crystallization analyses and structural determinations.

5 There is also a need to provide methods for identifying inhibitors of Hdm2 through structure-based drug design and for combining potential inhibitors with the crystals of Hdm2 and analyzing their binding. Further, there is a need to obtain Hdm2 protein samples that, when combined with potential inhibitors, are amenable to forming homogenous crystals.

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SUMMARY OF THE INVENTION

The present invention provides modified Hdm2 proteins that are amenable to crystallization and are soluble in *E. coli* extracts. The present invention further discloses a set of amino acid substitutions of the Hdm2 protein that improve its solubility and/or stability without compromising its ability to bind

15 p53. It is a further object of the present invention to provide a modified Hdm2 protein having an amino acid substitution at one or more of the seven sites defined in Table 1. In one embodiment, the modified Hdm2 protein comprises the amino acid of SEQ ID NO: 4, wherein one or more of the seven specific amino acid residues denoted as X₁-X₇ of SEQ ID NO: 4 differs from that of wild-type Hdm2(17-125) (SEQ ID NO: 2) or said amino acid sequence comprising one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4. In other embodiments, the modified Hdm2 protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 6, 8 10 and 12; or said amino acid sequence comprising one or more 20 conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4.

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The present invention further provides isolated and/or recombinant nucleic acids that encode the modified Hdm2 proteins of the present invention, as well as specific peptide fragments and fusion proteins thereof. In one 30 embodiment, the nucleic acid encodes a modified Hdm2 protein comprising the amino acid of SEQ ID NO: 4, wherein one or more of the seven specific amino acid residues denoted as X₁-X₇ of SEQ ID NO: 4 differs from that of wild-type

Hdm2(17-125) (SEQ ID NO: 2) or said amino acid sequence comprising one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4. In other embodiments, the nucleic acid encodes a modified Hdm2 protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 6, 8 10 and 12; or said amino acid sequence comprising one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4. In certain embodiments, the nucleic acid may comprise a nucleotide sequence selected from the group consisting of SEQ ID NOS: 5, 7, 9 and 11. The present invention further provides expression vectors that can comprise any of the nucleic acids of the present invention and a transcriptional control sequence. Preferably the nucleic acids of the present invention are operatively linked to a transcriptional control sequence in expression vectors. Host cells comprising the expression vectors are also part of the present invention. In one particular embodiment, the host cell is an *E coli* cell.

In addition, the present invention provides methods for producing the above-mentioned modified Hdm2 proteins. One such embodiment comprises culturing a host cell of the present invention that expresses a nucleic acid encoding a modified Hdm2 protein of the present invention, thereby producing the modified Hdm2 protein. Methods for purifying and/or obtaining the resulting recombinant modified Hdm2 proteins are also included in the present invention, as are the purified recombinant modified Hdm2 proteins.

The present invention further provides compounds that bind to Hdm2. In one such embodiment the compound is an acetylated tripeptide. In a particular embodiment of this type the compound is Ac-^{6Cl}WAC_{3c}E. In another embodiment the compound is Ac-^{6Br}WAC_{3c}E.

The present invention further provides protein-ligand complexes between the modified Hdm2 proteins of the present invention and their ligands. Preferred ligands in the complex are Ac-^{6Cl}WAC_{3c}E and Ac-^{6Br}WAC_{3c}E.

Crystals comprising a modified Hdm2 protein, and/or one of the protein-ligand complexes of the present invention, also are part of the present invention.

Preferably, such crystals effectively diffract X-rays for the determination of the atomic coordinates of the protein and/or of the protein-ligand complex to a resolution of greater than 5.0 Å (e.g., at least 3.0 Å, at least 2.5 Å, at least 2.0 Å, or at least 1.5 Å).

5 The invention provides a crystal comprising a polypeptide selected from (a) a modified Hdm2 protein, characterized by structural coordinates comprising a root mean square deviation of conserved residue backbone atoms of less than about 1.5 Å (e.g., less than about 1.0 Å, less than about 0.5 Å, or less than about 0.1 Å) when superimposed on backbone atoms described by structural

10 coordinates of Table 3 or (b) a modified Hdm2 protein characterized by structural coordinates comprising a root mean square deviation of conserved residue backbone atoms of less than about 1.5 Å (e.g., less than about 1.0 Å, less than about 0.5 Å, or less than about 0.1 Å) when superimposed on backbone atoms described by structural coordinates of Table 4. In certain embodiments, the

15 crystal may be complexed with a compound that binds to modified Hdm2 (e.g., SCH549128, Ac-^{6Cl}WAC_{3c}E or Ac-^{6Br}WAC_{3c}E)

The invention also provides the three-dimensional structure of the modified Hdm2 protein. In one embodiment, the three-dimensional structure is characterized by structural coordinates comprising a root mean square deviation of conserved residue backbone atoms of less than about 1.5 Å (e.g., less than about 1.0 Å, less than about 0.5 Å, or less than about 0.1 Å) when superimposed on backbone atoms described by structural coordinates of Table 3 or 4. The present invention further provides methods of using this three-dimensional structural information in drug discovery and/or to solve corresponding structures of Hdm2 homologues, other crystalline forms of Hdm2 mutants, and co-complexes of Hdm2 and its ligands.

In another aspect of the present invention, methods are provided for obtaining a crystal comprising a modified Hdm2 protein. In one embodiment, the crystal is obtained by vapor diffusion.

30 In another aspect, the present invention provides a crystalline form of Hdm2 protein that is amenable to ligand soaking experiments, which enables X-

ray crystallographic structural determinations to be performed on multiple Hdm2-ligand complexes in rapid succession.

The present invention further provides methods of obtaining a crystal comprising a protein-ligand complex between a ligand and a polypeptide

5 comprising a modified Hdm2 protein. The present invention further provides methods for exchanging ligands within a crystal.

In yet another aspect, the present invention provides a method for designing, selecting and/or optimizing a compound and then evaluating it for use as an inhibitor of Hdm2. One such embodiment comprises obtaining a set of

10 atomic coordinates that define the three-dimensional structure of the modified Hdm2 protein from a crystal of the present invention. In a related embodiment, a set of atomic coordinates that define the three-dimensional structure of the protein-ligand binding complex from a crystal of the present invention is obtained. In either case, a potential agent is then designed, selected or

15 optimized by performing structure-based drug design with the atomic coordinates obtained. Preferably, the design or selection is performed in conjunction with computer modeling.

In another aspect, the invention provides a method for evaluating the ability of a potential inhibitor to associate with Hdm2 comprising employing

20 computational means to perform a fitting operation between the potential inhibitor and the structure coordinates of Hdm2 and quantitating the association between the potential inhibitor and Hdm2.

A compound that is predicted to inhibit Hdm2 can be synthesized, if necessary, and subsequently contacted with Hdm2 or an active fragment

25 thereof. The activity of the compound is then determined by an assay that measures one or more of Hdm2's activities, as described above. A compound that is predicted to inhibit Hdm2 is identified as an inhibitor of Hdm2 when there is a decrease in the activity of Hdm2 in the presence of the agent relative to its absence.

30 In a related aspect of the present invention, a computer is provided that comprises a three-dimensional representation of a modified Hdm2 protein in

computer memory. One such computer comprises a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, comprising the atomic coordinates of Table 3 or 4. In another embodiment, the computer comprises a machine-readable data storage medium
5 comprising a data storage material encoded with machine-readable data, comprising the atomic coordinates of Table 3 or 4. In yet another embodiment, the computer comprises a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, comprising the atomic coordinates of Table 3 or 4. Preferably, the computer further comprises
10 a working memory for storing instructions for processing the machine-readable data, a central-processing unit coupled to the working memory and to the machine-readable data storage medium for processing the machine readable data into a three-dimensional representation of the modified Hdm2 protein and/or Hdm2 protein-ligand complex. More preferably, the computer includes a display
15 coupled to the central-processing unit for displaying the three-dimensional representation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides stable modified Hdm2 proteins produced by introducing an amino acid substitution into one or more of a unique set of
20 amino acid residues of Hdm2. The modified Hdm2 proteins of the present invention have an improved solubility and form novel crystals that heretofore were unattainable with the wild-type Hdm2 protein. The present invention further provides methods for generating and purifying these modified Hdm2 proteins. The modified Hdm2 proteins of the present invention may be used for the
25 structural determination of Hdm2 by X-ray crystallography and/or NMR.

Crystals of the modified Hdm2 protein and their resulting structures can be used to design high affinity inhibitors of Hdm2 that may be used in the treatment of cancer. Such drugs may be particularly useful to treat soft-tissue tumors, osteosarcomas and oesophageal carcinomas.

30 Structure-based drug design is the most efficient method for such drug development. In one common paradigm, a three dimensional structure is

determined for a protein, e.g., the modified Hdm2, and/or a corresponding protein-ligand complex. Potential antagonists (e.g., inhibitors and/or potential drugs) of the protein are then identified and/or designed with the aid of computer modeling [Bugg *et al.*, *Scientific American*, Dec.: 92-98 (1993); West *et al.*, *TIPS*, 5 16:67-74 (1995); Dunbrack *et al.*, *Folding & Design*, 2:27-42 (1997)]. The drug candidates are then selected and tested. The most promising drug candidates are identified and then combined with the protein in a crystalline protein-ligand complex. The three-dimensional structure of the protein-ligand complex is then determined, and new potential antagonists of the protein are identified and/or 10 designed with the aid of computer modeling. This process can then be continued in successive iterations until a lead drug candidate is identified.

Heretofore, the ability to perform structure based drug design with Hdm2 was severely hampered due to the lack of a crystalline form of the Hdm2 that is conducive for such studies. The expression and purification of a modified Hdm2 15 protein that when placed in a protein-ligand complex can form a monodisperse preparation, as disclosed herein, is therefore critical for the initiation of a structure based drug design program.

In addition, the present invention provides two specific ligands for Hdm2, the acetylated tripeptides, Ac-^{6Cl}WAC_{3c}E and Ac-^{6Br}WAC_{3c}E. Their precise 20 chemical structures are provided below. These tripeptides can be used to bind the modified Hdm2 proteins of the present invention to form a protein-ligand complex that is then crystallized. Such X-ray diffractable crystals can be used for structure based drug design to identify anti-tumor drugs.

Patches of hydrophobic amino acid residues on the surface of the Hdm2 25 protein were initially determined to be a critical factor leading to the relative insolubility of the wild-type Hdm2 protein. Interrupting these hydrophobic patches by replacing selected surface hydrophobic amino acid residues with hydrophilic amino acid alternatives were found to increase the solubility of the ensuing modified Hdm2 protein, as well as create new crystal contact sites.

The present invention therefore provides several approaches for 30 identifying appropriate hydrophobic amino acid residues to be replaced by more

hydrophilic alternatives. One such approach entails employing the Clustalw program to align the amino acid sequences of Hdm2 and Hdm4 analogs from a number of species to identify natural amino acid variations. In one particular alignment, protocol Hdm2 or Hdm4 sequences from the following species were used: *Brachydanio rerio* (Zebrafish), *Canis familiaris* (Dog), *Equus caballus* (Horse), *Homo sapiens* (Human), *Mesocricetus auratus* (Golden Hamster), *Mus musculus* (Mouse), *Xenopus laevis* (African Clawed Frog) and *Gallus gallus* (Chicken). Seven Hdm2 surface amino acid residues in the hydrophobic patches of Hdm2 were identified and the preferred alternative amino acids at those sites noted, see Table 1 below. In a related approach, the seven amino acid substitutions were selected to specifically increase Hdm2 solubility, see modified Hdm2 (HK₅) having the amino acid sequence of SEQ ID NO: 12.

In still another approach, the amino acid sequences of Hdm2 and XMdm2 were compared to identify the positions of surface, solvent exposed, hydrophobic amino acid residues of the human protein that were occupied by more hydrophilic residues in the corresponding *Xenopus laevis* amino acid sequence. From this protein surface analysis, four potential amino acid substitutions were chosen: Human: *Xenopus laevis*: F55Y, Y76H, Y104S, V109S.

In accordance with the present invention, there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And Translation* [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; *Immobilized Cells And Enzymes* [IRL Press, (1986)]; B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1996) (herein "Ausubel et al., 1996").

As used herein the following terms shall have the definitions set out below:

As used herein the term "polypeptide" is used interchangeably with the term "protein" and is further meant to encompass peptides. Therefore, as used

5 herein, a polypeptide is a polymer of two or more amino acids joined together by peptide linkages. Preferably, a polypeptide is a polymer comprising twenty or more amino acid residues joined together by peptide linkages, whereas a peptide comprises five to twenty amino acid residues joined together by peptide linkages.

10 As used herein a polypeptide "consisting essentially of" or that "consists essentially of" a specified amino acid sequence is a polypeptide that

- (i) retains an important characteristic of the polypeptide comprising that amino acid sequence, e.g., the ability to bind p53 and/or act as a ligase for conjugating ubiquitin to p53, and

15 (ii) further comprises the identical amino acid sequence, except it consists of plus or minus 10% (or a lower percentage), and preferably plus or minus 5% (or a lower percentage) of the amino acid residues. In a particular embodiment, additional amino acid residues included as part of the polypeptide are part of a linked Tag, such as a C-terminal His₆ Tag.

20 The term "generic Mdm2" is used herein to refer to the double minute 2 polypeptide from any species. When used by itself, Mdm2 refers to mouse Mdm2. When used with another species name appended to it, it refers to the Mdm2 ortholog from the named species, e.g., Xenopus Mdm2 refers to the Mdm2 ortholog from Xenopus. "Mdm2" also encompasses modified forms thereof.

25 As used herein the terms "human Mdm2" and "Hdm2" are used interchangeably to denote the human ortholog of Mdm2. "Hdm2" also encompasses modified forms thereof. Hdm2 has the GenBank accession number of M92424. Mouse Mdm2 has the GenBank accession number of X58876 [see also, published U.S. patent application 2002/0045192, published April 18, 2002,

30 the contents of which are hereby incorporated by reference in their entirety.] The amino acid sequences provided herein correspond to amino acid residues 17-

125 of the full-length Hdm2 protein and retain their numerical designation from that full-length sequence. Therefore, the first amino acid residue of the wild-type Hdm2(17-125) sequence, SEQ ID NO: 2, corresponds to amino acid 17 of the full-length Hdm2 protein.

5 As used herein a “modified Hdm2” is identical to the wild-type Hdm2(17-125) except it has at least one amino acid substitution, *i.e.*, it has one or more amino acid substitutions. Furthermore, a modified Hdm2 of the present invention comprises an amino acid substitution at one or more of the seven positions listed in Table 1 and denoted in SEQ ID NO: 4. Preferably, that amino acid

10 substitution is one that is specifically defined in Table 1 (and denoted in SEQ ID NO: 4). It is also preferable that a modified Hdm2 protein of the present invention comprises 109 amino acid residues and that the positions of these 109 residues correspond to amino acid residues 17-125 of the full-length wild-type Hdm2 protein.

15 As used herein, a “conservative amino acid substitution” is the substitution of a functionally equivalent amino acid for an amino acid within the sequence of Hdm2. The conservative amino acid substitution can be at any position in the Hdm2 sequence except for the seven positions identified in Table 1, for which the alternatives are specifically defined. In general, a functionally equivalent

20 amino acid is one having a similar polarity and/or molecular properties for the amino acid within the sequence. Specifically, an amino acid substitute may be selected from other members of the class to which the amino acid belongs. The class of nonpolar amino acids includes alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The class of polar neutral

25 amino acids includes glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The class of positively charged (basic) amino acids includes arginine, and lysine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Particularly preferred conserved amino acid exchanges are:

30 (a) Lys for Arg or *vice versa* such that a positive charge may be maintained;

(b) Glu for Asp or *vice versa* such that a negative charge may be maintained;

(c) Ser for Thr or *vice versa* such that a free -OH can be maintained;

(d) Gln for Asn or *vice versa* such that a free NH₂ can be maintained; and

5 (e) Ile for Leu or for Val or *vice versa* as roughly equivalent hydrophobic amino acids.

As used herein the term “specific peptide fragment” is a peptide that comprises at least six amino acid residues, and preferably at least twelve amino acid residues of a modified Hdm2 protein that differs from the corresponding 10 fragment of the wild-type Hdm2 by at least one amino acid residue. Furthermore, the different amino acid residue (or different residues) is located at a position that corresponds to one (or more) of the seven variable sites denoted in SEQ ID NO: 4 and listed Table 1 below.

As used herein the term “chimeric” protein is meant to include fusion 15 proteins. “Chimeric” proteins of the present invention comprise at least a portion of a non-Hdm2 protein or peptide joined via a peptide bond to at least a portion of an Hdm2 protein, preferably a modified Hdm2. Chimeric proteins can have additional structural, regulatory, and/or catalytic properties. As used herein a chimeric protein can contain multiple additions to at least a portion of a modified 20 Hdm2 protein, e.g., it can comprise both a His₆Tag and a signal sequence. In a particular embodiment the chimeric protein functions as a means of detecting and/or isolating the polypeptide or fragment thereof after a recombinant nucleic acid encoding the modified Hdm2 protein or fragment thereof is expressed. Non-Hdm2 amino acid sequences are preferably either amino- or carboxy- 25 terminal to the modified Hdm2 sequence.

As used herein, DNA and protein sequence percent identity can be determined using C, MacVector 6.0.1, Vector NTI (Informax, Inc. MD), Oxford Molecular Group PLC (1996) and the Clustal W algorithm with the alignment default parameters, and default parameters for identity. These commercially 30 available programs can also be used to determine sequence similarity using the same or analogous default parameters. Alternatively, an Advanced Blast search

under the default filter conditions can be used, e.g., using the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program using the default parameters.

As used herein a "nucleic acid" refers to the phosphate ester polymeric

5 form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-

10 RNA and RNA-RNA helices are possible. When referring to a nucleic acid that is double stranded both the "sense" strand and the complementary "antisense" strand are intended to be included. Thus a nucleic acid that is hybridizable to SEQ ID NO: 1, for example, can be either hybridizable to the "sense" strand of SEQ ID NO: 1, which is particularly listed in the SEQUENCE LISTING, or to the

15 "antisense" strand which can be readily determined from that SEQUENCE LISTING.

A DNA "coding sequence" is a double-stranded DNA sequence that is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries

20 of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended

25 for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic

30 cells, polyadenylation signals are control sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which can then be trans-RNA spliced and translated into the protein encoded by the coding sequence.

A nucleic acid sequence is "operatively linked" to an expression control sequence when the expression control sequence controls or regulates the transcription and translation of that nucleic acid sequence. The term operatively linked includes having an appropriate start signal.

A "heterologous nucleotide sequence" as used herein is a nucleotide sequence that is added by recombinant methods to a nucleotide sequence encoding a modified Hdm2 of the present invention or encoding a fragment thereof, to form a nucleic acid that is not naturally formed in nature. Such nucleic acids can encode chimeric proteins. In addition, as used herein, a heterologous nucleotide sequence need not be a single contiguous nucleotide sequence, but can include multiple non-contiguous nucleotide sequences that have been combined with a nucleotide sequence encoding a modified Hdm2 protein of the present invention, or a portion thereof. A heterologous nucleotide sequence can comprise non-coding sequences including restriction sites, regulatory sites, promoters and the like. In still another embodiment the heterologous nucleotide can function as a means of detecting a nucleotide sequence of the present invention. The present invention provides heterologous nucleotide sequences that, when combined with nucleotide sequences encoding

a modified Hdm2 protein or a fragment thereof, are necessary and sufficient to encode all of the chimeric proteins of the present invention.

The phrase "binding to" in regard to a ligand binding to a polypeptide is used herein to include any or all such specific interactions that lead to a protein-ligand binding complex. This can include processes such as covalent, ionic (electrostatic and/or charged), hydrophobic and hydrogen bonding, but does not include non-specific associations such solvent preferences.

As used herein a "ligand" of a Mdm2 protein, e.g., a modified Hdm2 protein, is a compound that binds to the polypeptide in a protein-ligand binding complex. In a specific embodiment of the present invention the ligand inhibits the ability of the Mdm2 protein to bind p53 when the ligand is bound to the Mdm2 protein in a protein-ligand binding complex. In another embodiment, the ligand inhibits the ability of Mdm2 to act as an E3 ligase for conjugating ubiquitin to p53 when the ligand is bound to the Mdm2 protein in a protein-ligand binding complex. Such ligands may also be termed an "inhibitor".

As used herein, a "protein-ligand binding complex" or polypeptide-compound complex" is a specific association between a polypeptide and the compound that binds to it. In a preferred embodiment of the present invention, the ligand or compound is an inhibitor of the polypeptide. In a particular embodiment of this type, the binding of the inhibitor to the polypeptide occurs at the active site of the polypeptide.

As used herein "incubating a ligand with a crystal" is used interchangeably with "soaking a crystal with a ligand". Incubating a ligand with a crystal is the contacting of a ligand with a crystal of a polypeptide under the appropriate conditions and for a sufficient time period (e.g., hours to several days) for the ligand to bind to the crystalline polypeptide and form a crystalline protein-ligand complex. Such incubating can further include contacting an excess of a substitute ligand with a crystal of a protein-ligand complex under the appropriate conditions and for a sufficient time period (e.g., hours to several days) for the substitute ligand to replace the initial ligand and form the new crystalline protein-ligand complex.

As used herein the terms "displacing", "replacing", and "exchanging" are used interchangeably in regard to the substitution of one ligand in a protein-ligand complex for another.

As used herein an "excess of a substitute ligand" is an amount of that

5 ligand that is sufficient to replace 80% or more, and preferably 90% or more, of the initial ligand in a protein-ligand complex. In a particular embodiment of this type, the concentration of the substitute ligand is about ten-fold higher than the concentration of the protein-ligand complex. In a preferred embodiment, the concentration of the substitute ligand is about one hundred-fold higher than the

10 concentration of the protein-ligand complex.

As used herein the term "X-ray diffractable crystal" is a crystal of a compound, *e.g.*, a protein that yields a discernable diffraction pattern when subjected to 0.5 to 2.5 Å incident X-ray radiation.

As used herein an "X-ray quality crystal" is an X-ray diffractable crystal

15 that can yield meaningful structural data of its crystalline composition when subjected to X-ray crystallographic analysis.

As used herein, and unless otherwise specified, the terms "agent", "potential drug", "compound", or "test compound" are used interchangeably, and refer to chemicals that have or potentially have a use as a modulator of the

20 activity of Mdm2. Preferably the modulator is an inhibitor of the binding complex formed between p53 and Mdm2. Preferably such agents include drugs for the treatment or prevention of a disease and/or condition involving the p53 transcription factor, *e.g.*, cancer. Therefore, such agents may be used, as described herein, in drug assays and drug screens and the like.

25 As used herein a "small organic molecule" is an organic compound [or organic compound complexed with an inorganic compound (*e.g.*, metal)] that has a molecular weight of less than 3 Kd.

As used herein the terms "approximately" and "about" are used to signify that a value is within twenty percent of the indicated value *i.e.*, an amino acid

30 sequence containing "approximately" 110 amino acid residues can contain between 88 and 132 amino acid residues.

As used herein the phrases "structure based rational drug design", "structure based drug design" and "structure assisted drug design" are used interchangeably. These phrases are meant to convey a particular method of identifying and/or designing a ligand (preferably an inhibitor) for a specific target 5 protein that includes the use of the three-dimensional structure of that protein and/or its corresponding protein-ligand complex.

Nucleic Acids Encoding Mdm2 Proteins

The nucleic acids can further comprise heterologous nucleotide sequences. In one embodiment, the nucleic acid encodes a modified Hdm2 10 protein comprising the amino acid of SEQ ID NO: 4, wherein one or more of the seven specific amino acid residues denoted as X₁-X₇ of SEQ ID NO: 4 differs from that of wild-type Hdm2(17-125) (SEQ ID NO: 2). In a particular embodiment of this type the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 3, wherein at least one nucleotide of one of the codons encoding 15 one or more of the seven specific amino acid residues denoted as X₁-X₇ of SEQ ID NO: 4 differs from the nucleic acid sequence of wild-type Hdm2(17-125) (SEQ ID NO: 1) and wherein said codon encodes a different amino acid from that of wild-type Hdm2(17-125) (SEQ ID NO: 2).

In one embodiment, the nucleic acid encodes a modified Hdm2 protein 20 comprising the amino acid sequence of SEQ ID NO: 6. In a particular embodiment of this type, the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 5. In another embodiment, the nucleic acid encodes a modified Hdm2 protein comprising the amino acid sequence of SEQ ID NO: 8. In a particular embodiment of this type, the nucleic acid comprises the nucleotide 25 sequence of SEQ ID NO: 7. In still another embodiment, the nucleic acid encodes a modified Hdm2 protein comprising the amino acid sequence of SEQ ID NO: 10. In a particular embodiment of this type, the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 9. In yet another embodiment, the nucleic acid encodes a modified Hdm2 protein comprising the amino acid 30 sequence of SEQ ID NO: 12. In a particular embodiment of this type, the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 11. Nucleic acids that consist of the nucleotide sequences that encode the modified Hdm2 proteins of

the present invention or that consist of nucleotide sequences that encode specific peptide fragments of those proteins are also provided. The present invention also includes those nucleic acids that encode a modified Hdm2 comprising one or more conservative amino acid substitutions. In certain 5 embodiments, there may be 1-11 conservative amino acid substitutions, preferably 1, 2 or 3 conservative amino acid substitutions. In a related embodiment, the present invention provides nucleic acids that further comprise a heterologous nucleotide sequence.

Obtaining and/or constructing a cDNA that encodes a Mdm2 protein, 10 including Hdm2 proteins and the modified Hdm2 proteins of the present invention, facilitates the production of the large quantities of protein required to perform standard enzyme assays and/or X-ray crystallographic analysis.

The present invention provides specific nucleic acid constructs that allow for the expression and isolation of large quantities of stable and active modified 15 Hmd2 proteins. These nucleic acids can further contain heterologous nucleotide sequences. To express a recombinant protein of the present invention in a host cell, an expression vector can be constructed comprising the corresponding cDNA. The present invention therefore, provides expression vectors containing nucleic acids encoding the modified Hmd2 proteins of the present invention. 20 Due to the degeneracy of nucleotide coding sequences, other DNA sequences that encode the same or substantially the same amino acid sequence as a nucleic acid encoding a modified Hmd2 protein of the present invention may be used in the practice of the present invention. These include, but are not limited to, allelic genes, homologous genes from other species, which are altered by the 25 substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Host cells comprising the expression vectors of the present invention are also provided. One particular host cell, an *E. coli* cell, is specifically exemplified below.

General methods for the cloning of cDNAs and expression of their 30 corresponding recombinant proteins have been described [see Sambrook and Russell, *Molecular Cloning, A Laboratory Manual, 3rd edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor L.I. (2000)]. The particular methodology

used herein is described in Example 1 below. Preferably, all of the nucleic acid constructs of the present invention are sequence confirmed.

Any technique for mutagenesis known in the art can be used to convert the native (wild-type) Hdm2 to a modified Hdm2 of the present invention,

5 including but not limited to, *in vitro* site-directed mutagenesis [Hutchinson *et al.*, *J. Biol. Chem.*, **253**:6551 (1978); Zoller and Smith, *DNA*, **3**:479-488 (1984);
Oliphant *et al.*, *Gene*, **44**:177 (1986); Hutchinson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **83**:710 (1986)]. The use of TAB@ linkers (Pharmacia), etc. and PCR techniques also can be employed for site directed mutagenesis [see Higuchi,
10 "Using PCR to Engineer DNA", in PCR Technology: Principles and Applications for DNA Amplification, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70
(1989)].

Preferably mutagenesis (*i.e.*, modification) of an Hdm2 transcript is performed in a two step process [Wang and Malcolm, *BioTechniques* **26**:680-
15 682 (1999)]. In Examples 2 and 3 below, two extension reactions were performed in separate tubes in the first stage: (i) one containing the forward primer, and (ii) the other containing the reverse primer. After two cycles, the two reactions are mixed and the standard QuickChange mutagenesis procedure was carried out for an additional 18 cycles. Following amplification, the parental
20 strand was digested with 1Unit of Dpn1 for 2 hours and an aliquot was transformed into DH5-alpha cells [GeneWiz, New York, N.Y.]. The pET32 Xa/LIC-hdm2 (17-125) vector was used as a template.

When the modified Hdm2 has three amino acid substitutions, as described in the Example 1 below, the GENETAILOR Site-directed Mutagenesis system (Invitrogen, Carlsbad, CA, USA) was used with the pET32 Xa/LIC-hdm2 (17-125) as the template. In this case the GENETAILOR mutagenesis was performed according to the manufacturer's instruction manual, with the lone exception being the use of the *pfu* turbo DNA polymerase.

The Modified Hdm2 Proteins

30 In one embodiment, the modified Hdm2 polypeptide of the invention comprises the amino acid sequence SEQ ID NO: 4, wherein one or more of the

seven specific amino acid residues denoted as X₁-X₇ of SEQ ID NO: 4 differs from that of wild-type Hdm2(17-125) (SEQ ID NO: 2). In another embodiment, the modified Hdm2 polypeptide comprises one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4. In certain
5 embodiments, there may be 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 conservative amino acid substitutions in the sequence at sites other than those indicated in Table 1. In preferred embodiments, there may be 1, 2 or 3 conservative amino acid substitutions at sites other than those indicated in Table 1.

In particular embodiments, the amino acid residues at all seven of the
10 variable positions in the amino acid sequence of SEQ ID NO: 4 are different than that of SEQ ID NO: 2. In a specific embodiment of this type, the modified Hdm2 protein is the Hdm2(HK5) comprising the amino acid sequence of SEQ ID NO: 12 or said modified Hdm2 protein comprising the amino acid sequence of SEQ ID NO: 12 comprising one or more conservative amino acid substitutions at sites
15 other than that of X₁-X₇ of SEQ ID NO: 4 as described above. In other embodiments, the modified Hdm2 protein comprises the amino acid sequence SEQ ID NO: 4 wherein the amino acid residues at six, five, four or three of the seven variable positions (X₁-X₇) in the amino acid sequence of SEQ ID NO: 4 are different than that of SEQ ID NO: 2. In certain embodiments, the modified
20 Hdm2 protein comprises one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4.

In yet another embodiment, the amino acid residues at two of the seven variable positions in the amino acid sequence of SEQ ID NO: 4 are different than that of SEQ ID NO: 2. In a particular embodiment of this type the modified Hdm2
25 protein is Hdm2(F55Y/Y76H) protein comprising the amino acid sequence of SEQ ID NO: 10 or said modified Hdm2 protein comprising one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4. In another embodiment, only one of the amino acid residues at the seven variable positions in the amino acid sequence of SEQ ID NO: 4 is different than
30 that of SEQ ID NO: 2. In a particular embodiment of this type, the modified Hdm2 protein is the Hdm2(F55Y) protein comprising the amino acid sequence of SEQ ID NO: 8 or said modified Hdm2 protein comprising one or more

conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4. In another particular embodiment, the modified Hdm2 protein is Hdm2(Y76H) protein comprising the amino acid sequence of SEQ ID NO: 6 or said modified Hdm2 protein comprising one or more conservative amino acid 5 substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4.

The present invention further provides a modified Hdm2 protein consisting of, or consisting essentially of, SEQ ID NOS: 4, wherein one or more of the seven specific amino acid residues denoted as X₁-X₇ of SEQ ID NO: 4 differs from that of wild-type Hdm2(17-125) (SEQ ID NO: 2). In certain embodiments, 10 said modified Hdm2 protein comprises one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID NO: 4. A modified Hdm2 protein is further provided that consists of, or consists essentially of, SEQ ID NOS: 6, 8, 10 or 12, or said modified Hdm2 protein comprising one or more conservative amino acid substitutions at sites other than that of X₁-X₇ of SEQ ID 15 NO: 4.

Fusion proteins that comprise the modified Hdm2 proteins of the present invention are also provided, as well as specific peptide fragments of those proteins.

In one such embodiment, the modified Hdm2 protein comprises the amino 20 acid sequence of SEQ ID NO: 4. In a preferred embodiment, the modified Hdm2 protein comprises the amino acid sequence of SEQ ID NO: 6. In one embodiment the compound is Ac-^{6Br}WAC_{3c}E. In a preferred embodiment, the compound is Ac-^{6Cl}WAC_{3c}E.

The amino acid sequences of the wild-type (WT) Hdm2 and the following 25 modified Hdm2 proteins corresponding to amino acid residues 17-125 of the full-length wild-type Hdm2 are provided below:

WT Hdm2(17-125): SEQ ID NO: 2

SQIPASEQETLVRPKPLLKLLKSVGAQKDVTMKEVLFYLGQYIMTKRL
YDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIVRNLVVVNQQESSDSGT
30 SVSEN

Modified Hdm2 (X₁-X₇): SEQ ID NO: 4

SQIPASEQETX₁VRPKPX₂LLKLLKSVGAAQKDTYTMKEVLX₃YLGQYIMTK
 RLYDEKQQHIVX₄CSNDX₅LGDLFGVX₆SFSVKEHRKIYTMIX₇RNLVVVNQQES
 SDSGTSVSEN

5 Modified Hdm2 (Y76H): SEQ ID NO: 6

SQIPASEQETLVRPKPLLLKLLKSVGAAQKDTYTMKEVLFYLGQYIMTKRL
 YDEKQQHIVHCSNDLLGDLFGVPSFSVKEHRKIYTMIIYRNLVVVNQQESSDSG
 TSVSEN

Modified Hdm2 (F55Y): SEQ ID NO: 8

10 SQIPASEQETLVRPKPLLLKLLKSVGAAQKDTYTMKEVLYYLGQYIMTKRL
 YDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIIYRNLVVVNQQESSDSGT
 SVSEN

Modified Hdm2 (F55Y/Y76H): SEQ ID NO: 10

15 SQIPASEQETLVRPKPLLLKLLKSVGAAQKDTYTMKEVLYYLGQYIMTKRL
 YDEKQQHIVHCSNDLLGDLFGVPSFSVKEHRKIYTMIIYRNLVVVNQQESSDSG
 TSVSEN

Modified Hdm2 (HK₅): SEQ ID NO: 12

20 SQIPASEQETKVRPKPKLLKLLKSVGAAQKDTYTMKEVLHYLGQYIMTKR
 LYDEKQQHIVKCSNDKLGDLFGVKSFVKEHRKIYTMIIYRNLVVVNQQESSSDS
 GTSVSEN

Modified Hdm2 (HK₅) as indicated above has six amino acid substitutions (L27K, L33K, F55H, Y76K, L81K, and P89K) selected to specifically increase Hdm2 solubility; only two are derived from natural variants.

Column 1 of Table 1 denotes the seven variable amino acid positions in the sequence of SEQ ID NO: 4, and presents the numbering of the amino acid positions in relation to both the full-length wild-type Hdm2 and the corresponding numbering of the wild-type Hdm2(17-125) having the amino acid sequence of SEQ ID NO: 2 (in parentheses). The seven defined positions in SEQ ID NO: 4 in which the amino acid residue can specifically vary are denoted as X₁-X₇ (column

2). The amino acid residues in the full-length wild-type Hmd2 occupying those seven positions are listed in column 3. All of the natural variants identified by the Clustalw alignment of the amino acid sequences of Hdm2 and Hdm4 analogs using *Brachydanio rerio* (Zebrafish), *Canis familiaris* (Dog), *Equus caballus*

5 (*Horse*), *Homo sapiens* (Human), *Mesocricetus auratus* (Golden Hamster), *Mus musculus* (Mouse), *Xenopus laevis* (African Clawed Frog) and *Gallus gallus* (Chicken) are provided in column 4. All acceptable amino acids are listed in Column 5, including the amino acid residues for the seven respective positions of the wild-type Hmd2, which are in bold.

10 Table 1: Amino Acid Substitutions for SEQ ID NO: 4

<u>A.A. Position</u>	<u>A.A. Name</u>	<u>Hdm2</u>	<u>Species Variants</u>	<u>Acceptable Variants</u>
27 (11)	X ₁	L27	Q	L , K, R, Q, E, D, S
33 (17)	X ₂	L33	Q	L , K, R, Q, E, D, S
55 (39)	X ₃	F55	L , Y, H	F , H, Y, K, R, Q, E, D, S
76 (60)	X ₄	Y76	H	Y , H, K, R, Q, E, D, S
81 (65)	X ₅	L81	A, P, C	L , K, R, Q, E, D, S, P, A
89 (73)	X ₆	P89	K, V, Q, T	P , K, R, Q, E, D, S
104 (88)	X ₇	Y104	I, N, R, S	Y , K, R, Q, E, D, S, N

In addition, the modified Hdm2 proteins of the present invention may include conservative amino acid substitutions relative to the wild type sequence of Hdm2 (other than those at the seven positions identified in Table 1, for which 15 the alternatives are specifically defined). In general, there are no more than 11 conservative amino acid substitutions (e.g., no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 conservative amino acid substitutions). In a preferred embodiment, there are no more than 3 conservative amino acid substitutions (0, 1, 2 or 3).

All of the modified Hdm2 proteins of the present invention also can be part 20 of a chimeric protein. In a specific embodiment, a chimeric modified Hdm2 protein is expressed in a prokaryotic cell. Such a chimeric protein can be a fusion protein used to isolate a modified Hdm2 protein of the present invention,

through the use of an affinity column that is specific for the protein fused to the modified Hdm2 protein. Examples of such fusion proteins include: a glutathione-S-transferase (GST) fusion protein, a maltose-binding protein (MBP) fusion protein, a FLAG-tagged fusion protein, or a poly-histidine-tagged fusion protein.

5 Specific linker sequences such as a Ser-Gly linker can also be part of such a fusion protein. A chimeric modified Hdm2 protein of the present invention also can be expressed in a eukaryotic cell.

Expression of a chimeric, modified Hdm2 protein, or fragment thereof, as a fusion protein can facilitate stable expression, and/or allow for purification

10 based on the properties of the fusion partner. Thus the purification of the recombinant polypeptides of the present invention can be simplified through the use of fusion proteins having affinity Tags. For example, GST binds glutathione conjugated to a solid support matrix, MBP binds to a maltose matrix, and poly-histidine chelates to a Ni-chelation support matrix [see Hochuli *et al.*,
15 *Biotechnology* 6:1321-1325 (1998)]. The fusion protein can be eluted from the specific matrix with appropriate buffers, or by treating with a protease that is specific for a cleavage site that has been genetically engineered in between the modified Hdm2 protein and its fusion partner. Alternatively, a modified Hdm2 protein can be combined with a marker protein such as green fluorescent protein
20 [Waldo *et al.*, *Nature Biotech.* 17:691-695 (1999); U.S. Patent No. 5,625,048 and WO 97/26333].

Alternatively or in addition, other column chromatography steps (e.g., gel filtration, ion exchange, affinity chromatography etc.) can be used to purify the recombinant modified Hdm2 proteins of the present invention. In many cases,

25 such column chromatography steps employ high performance liquid chromatography or analogous methods in place of the more classical gravity-based procedures. The specific details for the preferred purification procedure of the modified Hdm2 proteins of the present invention are provided in Example 1 below.

30 In addition, the modified Hdm2 proteins of the present invention and proteins thereof including, specific peptide fragment thereof can be chemically

synthesized [see e.g., Synthetic Peptides: A User's Guide, W.H.Freeman & Co., New York, N.Y., pp. 382, Grant, ed. (1992)].

Crystallization of Protein

The modified Hdm2 proteins result in novel crystal forms not obtained with
5 the wild-type protein. In one embodiment, the modified Hdm2 protein may be
crystallized in the presence of one of a variety of different compounds (e.g., an
small molecule inhibitor or a peptide from p53). Further, a compound complexed
to modified Hdm2 may be exchanged in the crystal by a second compound (e.g.,
a potential inhibitor) by soaking the crystal in a solution containing the second
10 compound. These crystals and the resulting structures can be used to obtain a
detailed view of various inhibitors that bind to Hdm2, providing a basis for further
design of potent inhibitors of Hdm2 to be used as anticancer agents.

Crystallization may be accomplished by using any of the known methods
in the art (Giegé, et al., (1994) Acta Crystallogr. D50: 339-350; McPherson,
15 (1990) Eur. J. Biochem. 189: 1-23). Such techniques include microbatch,
hanging drop, seeding and dialysis. Preferably, hanging-drop vapor diffusion
(McPherson, (1976) J. Biol. Chem. 251: 6300 -6303) or microbatch methods
(Chayen (1997) Structure 5: 1269-1274) are used. In each of these methods, it
is important to promote continued crystal growth after nucleation by maintaining
20 a supersaturated solution. In the microbatch method, polypeptide is mixed with
precipitants to achieve supersaturation, the vessel is sealed and set aside until
crystals appear. In the dialysis method, polypeptide is retained in a sealed
dialysis membrane that is placed into a solution containing precipitant.
Equilibration across the membrane increases the precipitant concentration
25 thereby causing the polypeptide to reach supersaturation levels. It is desirable
to use a modified Hdm2 protein preparation having a concentration of at least
about 1 mg/mL and preferably about 5 mg/mL to about 60 mg/mL, more
preferably about 20 mg/mL to about 50 mg/mL, even more preferably about 30
mg/mL to about 40 mg/mL. Crystallization may be achieved in precipitant
30 solutions containing polyethylene glycol 1000-20,000 (PEG; average molecular
weight ranging from about 1000 to about 20,000 Da), preferably about 2000 to
about 6000 Da, more preferably about 5000 Da, with concentrations ranging

from about 10% to about 50% (w/v). It may also be desirable to include a protein stabilizing agent. If glycerol is chosen as the protein stabilizing agent, it is preferably provided at a concentration ranging from about 0.5% to about 20%. A suitable salt, such as magnesium chloride, potassium chloride, sodium chloride, 5 lithium chloride or sodium citrate may also be desirable in the precipitant solution, preferably in a concentration ranging from about 1mM to about 2000 mM. The precipitant is preferably buffered to a pH of from about 6.5 to about 9.5, preferably about 7.5-8.5. Specific buffers useful in the precipitant solution may vary and are well-known in the art (Scopes, Protein Purification: Principles 10 and Practice, Third ed., (1994) Springer-Verlag, New York). Examples of useful buffers include, but are not limited to, Hepes, Tris, MES and acetate. Crystals routinely grow at a wide range of temperatures. It is, however, preferred that crystals form at temperatures between about 2°C and about 26°C.

The crystals of the present invention have a wide range of uses. For 15 example, high quality crystals are suitable for X-ray or neutron diffraction analysis to determine the three dimensional structure of Hdm2 and in particular to assist in the identification of the protein's effector sites. Knowledge of these sites and solvent accessible residues allow structure-based design and construction of agonists and antagonists for Hdm2.

20 In addition, crystallization itself can be used as a purification method. In some instances, a polypeptide or protein crystallizes from a heterogeneous mixture into crystals. Isolation of such crystals by filtration and/or centrifugation, followed by redissolving the polypeptide affords a purified solution suitable for use in growing high-quality crystals that are preferred for diffraction analysis.

25 Once a crystal of the present invention is grown, X-ray diffraction data can be collected. One method for determining structure with X-ray diffraction data includes use of synchrotron radiation, under standard cryogenic condition; however, alternative methods may also be used. For example, crystals can be characterized by using X-rays produced by a conventional source, such as a 30 sealed tube or a rotating anode. Methods of characterization include, but are not limited to, precession photography, oscillation photography and diffractometer data collection.

The crystallizable compositions provided by this invention may be amenable to X-ray crystallography for providing the three-dimensional structure of a Hdm2 polypeptide. The present invention includes crystals which effectively diffract X-rays for the determination of the atomic coordinates of Hdm2 to a

5 resolution of greater than about 5.0 Ångströms (e.g., about 4.5 Å, about 4.0 Å, about 3 Å, about 2.5 Å, about 2 Å, about 1 Å, about 0.5 Å, about 0.1 Å), preferably greater than about 4.0 Ångströms (e.g., about 3 Å, about 2.5 Å, about 2 Å, about 1 Å, about 0.5 Å, about 0.1 Å), more preferably greater than about 2.8 Ångströms (e.g., about 2.5 Å, about 2 Å, about 1 Å, about 0.5 Å, about 0.1 Å)

10 and most preferably greater than about 2.0 Ångströms (e.g., about 1.5 Å, about 1.0 Å, about 0.5 Å, about 0.1 Å).

The present invention includes Hdm2 crystals whose three-dimensional structure is described by the structure coordinates set forth in Table 3 or 4. The scope of the present invention also includes crystals which possess structural

15 coordinates which are similar to those set forth in Table 3 or 4; preferably, the crystals or the soluble polypeptides which are used to form the crystals exhibit Hdm2 catalytic activity and/or p53 binding (see above). In some embodiments, the crystal comprises a polypeptide that comprises the amino acid sequence of SEQ ID NO: 4, wherein one or more of the seven specific amino acid residues

20 denoted as X₁-X₇ of SEQ ID NO: 4 differs from that of wild-type Hdm2(17-125) (SEQ ID NO: 2). In other embodiments, the crystal comprises a polypeptide that comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 8 and 12. In another embodiment, the modified Hdm2 polypeptide comprises one or more conservative amino acid substitutions at sites other than

25 that of X₁-X₇ of SEQ ID NO: 4. Structural similarity between crystals is discussed in detail below.

The term "structure coordinates" refers to Cartesian coordinates derived from mathematical equations related to the patterns obtained on diffraction of a beam of X-rays by the atoms (scattering centers) of a molecule. The diffraction

30 data are used to calculate electron density maps and to establish the positions of the individual atoms of the molecule.

Those of skill in the art will understand that a set of structure coordinates for a protein or a protein complex or a portion thereof, is a relative set of points that define a shape in three dimensions. Thus, it is possible that an entirely different set of coordinates could define a similar or identical shape. Moreover,

5 slight variations in the individual coordinates will have little effect on overall shape.

The present invention includes crystals exhibiting structure coordinates which are similar to those set forth in Table 3 or 4 but for crystallographic permutations of the structure coordinates, fractionalization of the structure

10 coordinates, additions, subtractions, rotations or translations to sets of the structure coordinates or any combinations of the above.

Alternatively, modifications in the crystal structure due to mutations, additions, substitutions, and/or deletions of amino acids, or other changes in any of the components that make up the crystal may also account for variations in

15 structure coordinates. If such variations are within an acceptable standard error as compared to the coordinates of Table 3 or 4, the resulting three-dimensional shape is considered to be the same and, accordingly, the modified crystal is considered to be within the scope of the present invention.

Various computational analyses may be necessary to determine whether

20 a crystal is sufficiently similar to the crystals whose structural coordinates are set forth in Table 3 or 4 as to be considered the same. Such analyses may be carried out in current software applications, such as the Molecular Similarity application of QUANTA (Molecular Simulations Inc., San Diego, CA) version 4.1, and as described in the accompanying User's Guide.

25 The Molecular Similarity application permits comparisons between different structures, different conformations of the same structure, and different parts of the same structure. In general, the procedure used in Molecular Similarity to compare structures is divided into four steps: 1) input the structures to be compared; 2) define the atom equivalences in these structures; 3) perform

30 a fitting operation; and 4) analyze the results.

Each structure is identified by a name. One structure is identified as the target (i.e., the fixed structure); all remaining structures are working structures (i.e., moving structures). Since atom equivalency within QUANTA is defined by user input, for the purpose of this invention we will define equivalent atoms as

5 protein backbone atoms (N, CA, C and O) for all conserved residues between the two structures being compared.

When a rigid fitting method is used, the working structure is translated and rotated to obtain an optimum fit with the target structure. The fitting operation uses a least squares fitting algorithm that computes the optimum translation and

10 rotation to be applied to the moving structure, such that the root mean square difference of the fit over the specified pairs of equivalent atom is an absolute minimum. This number, given in Ångströms, is reported by QUANTA.

The term "root mean square deviation" (RMSD) is a commonly known term in the art which, in general, means the square root of the arithmetic mean of

15 the squares of the deviations from the mean distance of corresponding atoms. It is a way to express the deviation or variation from a trend or object.

For the purpose of this invention, any set of structure coordinates of a molecule that has a RMSD of conserved residue backbone atoms (N, CA, C, O) of less than about 2.0 Å when superimposed--using backbone atoms--on the

20 relevant structure coordinates of Table 3 or 4 are considered identical and are within the scope of the present invention. Preferably the crystal is a modified Hdm2 protein as defined above. Preferably, the root mean square deviation is less than about 1.5 Å, more preferably less than 1.0 Å, even more preferably, the root mean square deviation is less than about 0.5 Å and most preferably, the

25 root mean square deviation is less than about 0.1 Å.

The term "least squares" refers to a method based on the principle that the best estimate of a value is that in which the sum of the squares of the deviations of observed values is a minimum.

In one embodiment, the modified Hdm2 protein comprises the amino acid sequence of SEQ ID NO: 6. In a particular embodiment of this type, the crystal

30 has the space group of P2₁2₁2₁, having unit cell dimensions of: a = 41.1,

b = 66.1, c = 96.1 Angstroms: In a particular embodiment of this type, the modified Hdm2 protein comprises the amino acid sequence of SEQ ID NO: 10. In a particular embodiment of this type, the crystal has the space group of P2₁2₁2₁, having unit cell dimensions of: a = 38.0, b = 45.3, c = 64.0 Angstroms.

5 In a related embodiment, the crystal comprises a protein-ligand binding complex with the modified Hdm2 protein.

In accordance with the present invention, the structure coordinates of the Hdm2 polypeptide and portions thereof may be stored in a machine-readable storage medium. Such data may be used for a variety of purposes, such as drug discovery and X-ray crystallographic analysis of a protein crystal (e.g., for producing a three-dimensional representation of Hdm2). Accordingly, one aspect of this invention provides a machine-readable data storage medium comprising a data storage material encoded with the structure coordinates set forth in Table 3 or 4. The machine-readable data storage medium may also include any set of structure coordinates of a molecule that has a root mean square deviation of conserved residue backbone atoms (N, CA, C, O) of less than about 1.5 Å, preferably, less than about 1.0 Å, more preferably less than about 0.5 Å and even more preferably less than about 0.1 Å when superimposed--using backbone atoms--on the relevant structure coordinates of Table 3 or 4.

A computer system, useful in reading the machine readable data storage medium, includes a computer comprising a central processing unit ("CPU") and a memory storage device and is also within the scope of the present invention. In general, the computer system may be any computer with an operating system such as MS-DOS, PC-DOS, Windows, OS/2, Unix, Unix variant or MacOS. Particularly preferred computer systems are the Silicon Graphics Octane workstation or Compaq AlphaServer DS20. Other hardware systems and software packages will be known to those skilled in the art.

Input hardware coupled to the computer system by input line, may be implemented in a variety of ways. Machine-readable data of this invention may be input via the use of a modem or modems connected by a telephone line or a dedicated data line. Alternatively or additionally, the input hardware may

comprise CD-ROM drives or disk drives. A keyboard may also be used as an input device.

Output hardware, coupled to the computer system by output lines, may similarly be implemented by conventional devices. By way of example, output

5 hardware may include a display terminal (e.g., a cathode ray tube (CRT)) for displaying a graphical representation of the three dimensional structure of Hdm2 or a portion thereof using a program such as INSIGHT (Molecular Simulations Inc., San Diego, CA) or QUANTA as described herein. Output hardware might also include a printer, so that hard copy output may be produced, or a disk drive,

10 to store system output for later use. In preferred embodiments, the computer possesses a display which is displaying a three dimensional representation of Hdm2 or a fragment or homologue thereof.

In operation, the central processing unit (CPU) coordinates the use of the various input and output devices, coordinates data accesses from mass storage

15 and accesses to and from working memory, and determines the sequence of data processing steps. A number of programs may be used to process the machine-readable data of this invention. Such programs are discussed in reference to the computational methods of drug discovery as described herein. Specific references to components of the computer system are included as

20 appropriate throughout the following description of the data storage medium.

A magnetic data storage medium can be encoded with a machine-readable data by a computer system as described above. Storage medium may be, for example, a conventional floppy diskette or hard disk, having a suitable substrate, which may be conventional, and a suitable coating, which may be

25 conventional, on one or both sides, containing magnetic domains whose polarity or orientation can be altered magnetically. The magnetic domains of the coating of medium may be polarized or oriented so as to encode, in a manner which may be conventional, machine readable data, such as that described herein, for execution by a system as described herein. Storage medium may also have an

30 opening for receiving the spindle of a disk drive or other data storage device. Alternatively, an optically-readable data storage medium can be encoded with such machine-readable data, or a set of instructions. Medium can be a

conventional compact disk read only memory (CD-ROM) or a rewritable medium such as a magneto-optical disk which is optically readable and magneto-optically writable.

In general, in the case of CD-ROM, as is well known, disk coating is
5 reflective and is impressed with a plurality of pits to encode the machine-readable data. The arrangement of the pits is read by reflecting laser light off the surface of the coating. A protective coating, which preferably is substantially transparent, is provided on top of the coating.

In general, in the case of a magneto-optical disk, as is well known, disk
10 coating has no pits, but has a plurality of magnetic domains whose polarity or orientation can be changed magnetically when heated above a certain temperature, as by a laser. The orientation of the domains can be read by measuring the polarization of laser light reflected from the coating. The arrangement of the domains encodes the data as described above.

15 Structure Based Drug Design

The present invention permits the use of structure-based drug design techniques to design, select, and synthesize chemical entities, including inhibitory compounds that are capable of binding to a Hdm2 polypeptide. Also,
20 *de novo* and iterative drug design methods can be used to develop drugs from the structure of the Hdm2 crystals of this invention.

One particularly useful drug design technique enabled by this invention is structure-based drug design. Structure-based drug design is a method for optimizing associations between a protein and a compound by determining and evaluating the three-dimensional structures of successive sets of
25 protein/compound complexes.

Those skilled in the art will appreciate that association of natural ligands or substrates with the binding pockets of their corresponding receptors, enzymes or specific binding proteins is the basis of many biological mechanisms of action. The term "binding pocket", as used herein, may refer to any region of a molecule
30 or molecular complex, that, as a result of its shape, favorably associates with another chemical entity or compound. Similarly, drugs may exert their biological

effects through association with the binding pockets of receptors and/or specific binding proteins. Such association may occur with all or any part of the binding pockets. An understanding of such associations will help lead to the design of drugs having more favorable associations with the target protein, and thus,

5 improved biological effects. Therefore, this information is valuable in designing potential protein inhibitors, such as inhibitors of Hdm2.

In iterative structure-based drug design, crystals of a series of protein/compound complexes are obtained and then the three-dimensional structure of each complex is solved. Such an approach provides insight into the

10 association between the proteins and compounds of each complex. This is accomplished by selecting compounds with inhibitory activity, obtaining crystals of a new polypeptide, solving the three-dimensional structure of the polypeptide, and comparing the associations between the new protein and previously solved protein. By observing how changes in the compound affected the

15 protein/compound associations, these associations may be optimized.

In some cases, iterative structure-based drug design is carried out by forming successive protein-compound complexes and then crystallizing each new complex. Alternatively, a pre-formed protein crystal is soaked in the presence of an inhibitor or other binding compound, thereby forming a

20 protein/compound complex and obviating the need to crystallize each individual protein/compound complex. Advantageously, the modified Hdm2 crystals provided by this invention may be soaked in the presence of compounds, such as Hdm2 inhibitors, substrates or other ligands to provide novel Hdm2/compound crystal complexes. In one embodiment, the complexes may be

25 produced and screened using high throughput methods to quickly identify or design potential inhibitors of Hdm2.

The structure coordinates set forth in Table 3 or 4 can also be used to aid in obtaining structural information about another crystallized molecule or molecular complex. This may be achieved by any of a number of well-known

30 techniques, including molecular replacement.

The structure coordinates set forth in Table 3 or 4 can also be used for determining at least a portion of the three-dimensional structure of molecules or

molecular complexes which contain at least some structurally similar features to Hdm2. In particular, structural information about another crystallized molecule or molecular complex may be obtained by well-known techniques, including molecular replacement.

5 Therefore, another aspect of this invention provides a method of utilizing molecular replacement to obtain structural information about a crystallized molecule or molecular complex, whose structure is unknown, comprising the steps of generating an X-ray diffraction pattern from said crystallized molecule or molecular complex and applying crystallographic phases derived from at least a
10 portion of the structure coordinates set forth in Table 3 or 4 to the X-ray diffraction pattern to generate a three-dimensional electron density map of the molecule or molecular complex whose structure is unknown.

Once the structure coordinates of a protein crystal have been determined, they are useful in solving the structures of other crystals. In addition, the
15 structure of Hdm2 homologues may be determined from the structural coordinates of the present invention. For example, polypeptides may be crystallized and their structures elucidated by, for example, difference Fourier techniques and molecular replacement.

By using molecular replacement, all or part of the structure coordinates of
20 the Hdm2 polypeptide provided by this invention (and set forth in Table 3 or 4) can be used to determine the previously unknown structure of a crystallized molecule or molecular complex more quickly and efficiently than attempting to determine such information *ab initio*.

Molecular replacement provides an accurate estimation of the phases for
25 an unknown structure. Phases are a factor in equations used to solve crystal structures that cannot be measured experimentally. Obtaining accurate values for the phases, by methods other than molecular replacement, is a time-consuming process. However, when the crystal structure of a protein containing a homologous portion has been solved, the phases from the known structure
30 may provide a satisfactory estimate of the phases for the unknown structure.

Thus, this method involves generating a preliminary model of a molecule or molecular complex whose structure coordinates are unknown, by orienting

and positioning the relevant portion of the modified Hdm2 crystal according to Table 3 or 4 within the unit cell of the crystal of the unknown molecule or molecular complex so as best to account for the observed X-ray diffraction pattern amplitudes to generate an electron density map of the structure whose

5 coordinates are unknown. This, in turn, can be subjected to any well-known model building and structure refinement techniques to provide a final, accurate structure of the unknown crystallized molecule or molecular complex (Lattman, "Use of the Rotation and Translation Functions", in *Meth. Enzymol.*, 115: 55-77 (1985); Rossman, ed., "The Molecular Replacement Method", *Int. Sci. Rev. Ser.*,

10 No. 13, Gordon & Breach, New York (1972)).

Phase information from the structure coordinates of the present invention may be used to elucidate the structure of other crystals. For example, the structure of Hdm2 in complex with other atoms or molecules may be elucidated. Such complexes include, for example, those containing atoms soaked into or

15 cocrystallized within the crystal lattice. Other structures which can be elucidated using the phase information of the present invention include for example other proteases or homologues or mutants thereof having sufficient three-dimensional structure similarity to Hdm2 complex as to be solved using molecular replacement. Also, these protein molecules in a complex with a small molecule

20 substrate(s), inhibitor(s), transition state analog(s), product(s) or analog(s) of any of these may also be solved using the phase information of the present invention. Other complexes whose structure can be elucidated from the phase information of the present invention include a modified Hdm2 complexed with an inhibitor other than those presented herein. Complexes containing a

25 combination of the above molecules may also be solved using the phase information of the present invention.

The structure of any portion of any crystallized molecule or molecular complex that is sufficiently homologous to any portion of the modified Hdm2 protein can be solved by this method. The difference Fourier method simply

30 calculates an electron density map using phases calculated from the structure coordinates and observed diffraction amplitudes from a crystal of an unknown structure. This method is often used to solve structures of protein/ligand

complexes where the ligand is small and does not affect the crystal form significantly.

In a preferred embodiment, the method of molecular replacement is utilized to obtain structural information about a molecule wherein the molecule

5 comprises a Hdm2 polypeptide complex. The structure coordinates of modified Hdm2 provided by this invention are particularly useful in solving the structure of other crystal forms of Hdm2 polypeptide complexes. This approach enables the determination of the optimal sites for interaction between chemical entities, including interaction of candidate inhibitors with Hdm2.

10 Modified Hdm2 crystals may be studied using well-known X-ray diffraction techniques and may be refined versus X-ray data to 3Å resolution or better to an R_{free} value of about 0.40 or less using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; see e.g., Blundell & Johnson, *supra*; *Meth, Enzymol.*, vol. 114 & 115, H.W. Wyckoff et al., eds.,

15 Academic Press (1985)). This information may be used to optimize known Hdm2 inhibitors and to design new Hdm2 inhibitors.

Once a three-dimensional structure of a crystal comprising a modified Hdm2 protein in a protein-ligand complex is determined, the potential inhibitor of Hdm2 can be examined through the use of computer modeling using a docking

20 program such as GRAM, DOCK, or AUTODOCK [Dunbrack et al., *Folding & Design*, 2:27-42 (1997)]. This procedure can include computer fitting of potential inhibitors to the modified Hdm2 protein to ascertain how well the shape and the chemical structure of the potential modulator will interact with the modified Hdm2 protein [Bugg et al., *Scientific American*, Dec.:92-98 (1993); West et al., *TIBS*,

25 16:67-74 (1995)]. Computer programs can also be employed to estimate the attraction, repulsion, and steric hindrance of the modified Hdm2 protein with an inhibitor.

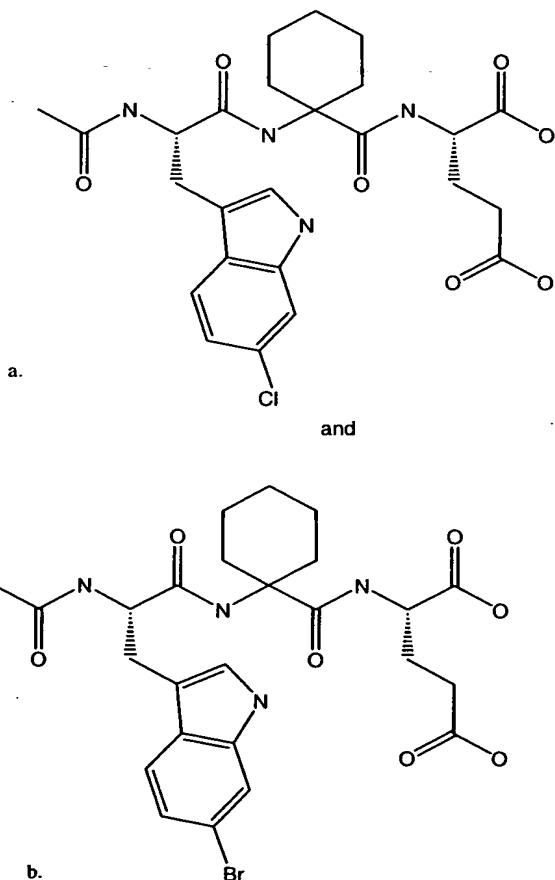
Generally the tighter the fit, the lower the steric hindrances, and the greater the attractive forces, the more potent the inhibitor, since these properties

30 are consistent with a tighter binding constant. Furthermore, the more specificity in the design of a potential drug the more likely that the drug will not interact as

well with other proteins. This will minimize potential side-effects due to unwanted interactions with other proteins.

Compounds that may be used initially have been discussed by Chene [Nature 3:102-109 (2003)]. In addition, the present invention discloses the acetylated tripeptides, (a) Ac-^{6Cl}WAC_{3c}E and (b) Ac-^{6Br}WAC_{3c}E, as shown respectively below, which can individually bind a modified Hmd2 protein in protein-ligand complex and form an X-ray diffractable crystal.

5



These ligands then can be systematically modified by computer modeling
10 programs until one or more promising potential analogs are identified. Such analysis has been shown to be effective in the development of HIV protease inhibitors [Lam *et al.*, *Science* 263:380-384 (1994); Wlodawer *et al.*, *Ann. Rev. Biochem.* 62:543-585 (1993); Appelt, *Perspectives in Drug Discovery and Design* 1:23-48 (1993); Erickson, *Perspectives in Drug Discovery and Design* 1:109-128 (1993)]. Alternatively, a potential inhibitor initially can be obtained by
15

screening a random peptide library or a chemical library. In the former case, a random peptide library can be produced by recombinant bacteriophage, for example, [Scott and Smith, *Science*, **249**:386-390 (1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci.*, **87**:6378-6382 (1990); Devlin *et al.*, *Science*, **249**:404-406 (1990)].

5 (1990)]. This approach may be particularly useful in this case since the natural binding partner for Mdm2 is the p53 protein. In any case, a peptide selected in this manner could then be systematically modified by computer modeling programs, as described above.

If a potential inhibitor is a small organic compound, it can be selected from
10 a library of chemicals, as are commercially available. Alternatively, the small organic compound may be synthesized *de novo*. Once obtained, the potential inhibitor can be further tested in a standard binding and/or functional assay with Hdm2, a modified Hdm2 protein or active fragments thereof.

For example, a binding assay can be performed following the attachment
15 of the Hdm2 protein to a solid support. Methods for placing Hdm2 protein on the solid support are well known in the art and include such things as linking biotin to the Hdm2 protein and linking avidin to the solid support. The solid support can be washed to remove unbound protein. A solution of a labeled potential inhibitor can be contacted with the solid support. The solid support is washed again to
20 remove the potential inhibitor not bound to the support. The amount of labeled potential inhibitor remaining with the solid support, and thereby bound to the Hdm2 protein can be determined. Alternatively, or in addition, the dissociation constant between the labeled potential inhibitor and the Hdm2 protein, for example, can be determined. Suitable labels for either the Hdm2 protein or the
25 potential inhibitor include, radioactive labels (e.g., ^{14}C , ^1H) and fluorescent labels such as fluorescein isothiocyanate (FITC).

In another embodiment, a Biacore machine can be used to determine the binding constant of the Hdm2 protein with a potential inhibitor [O'Shannessy *et al.* *Anal. Biochem.* **212**:457-468 (1993); Schuster *et al.*, *Nature* **365**:343-347 (1993)].

In addition, an inhibitor can be identified using an ELISA-based competition assay [Bottger *et al.*, *Oncogene* **13**:2141-2147 (1996); Bottger *et al.*, *J. Mol. Biol.* **269**:744-756 (1997)]. For example, the p53 protein or Hdm2-binding fragment thereof can be biotinylated and immobilized on a streptavidin-coated ELISA plate. Hdm2 is then incubated alone (in a control) or in the presence of a potential inhibitor. The Hdm2 solutions are then individually contacted with the immobilized p53 protein or Hdm2-binding fragment thereof. The binding of the Hdm2 is then determined, e.g., with a detectable anti-Hdm2 antibody. When the amount of Hdm2 detected is lower in the sample incubated with the potential inhibitor relative to the control, the potential inhibitor is identified as an inhibitor.

When a promising inhibitor is identified, a crystal comprising a protein-ligand complex of the inhibitor and a modified Hdm2 protein can be prepared. The three-dimensional structure of the resulting crystalline protein-ligand complex can then be determined by molecular replacement analysis, for example.

Molecular replacement involves the use of a known three-dimensional structure as a search model to determine the structure of a closely related molecule or protein-ligand complex in a different crystalline form. The measured X-ray diffraction properties of the new crystal are compared with the search model structure to compute the position and orientation of the protein in the new crystal. Computer programs that can be used include: X-PLOR [Brünger *et al.*, *Acta Crystallogr. A* **46**:585-593 (1990); Brünger *et al.*, *Acta Crystallogr. D Biol. Crystallogr.*, **54**:905-921 (1998)], CNS, (Crystallography and NMR System, a next level of XPLOR), and AMORE [Navaza, *Acta Crystallographica ASO*, **157**-163 (1994)]. Once the position and orientation are known, an electron density map can be calculated using the search model to provide X-ray phases. Thereafter, the electron density is inspected for structural differences and the search model is modified to conform to the new structure. Using this approach, it is possible to solve the three-dimensional structures of crystals of any protein-ligand complex of the modified Hdm2 protein.

For all of the drug screening assays described herein, further refinements to the structure of the drug will generally be necessary and can be made by the successive iterations of any and/or all of the steps provided by the particular drug screening assay and/or in combination with other such drug screening

5 assays.

A candidate drug selected by performing structure based drug design can then be assayed *in situ* and/or *in vivo*. For example, a candidate drug can be evaluated for cellular activity by incubating the candidate drug in cell cultures, e.g. using HCT-116 cells or OSA-CL cells, and then measuring its effect on

10 cellular proliferation and expression levels of proteins that are transcriptionally regulated by p53 such as p21waf1 and Hdm2 [Chene *et al.*, *J. Mol. Biol.* **299**:245-256 (2000)]. A candidate drug is identified as a drug if in the presence of the drug relative to in its absence, the amount of cellular proliferation decreases and/or the amount of a protein that is transcriptionally regulated by

15 p53 increases.

Indeed, methods of testing such potential candidate drugs in animal models are well known in the art. The potential drugs can be administered by a variety of ways including topically, orally, subcutaneously, or intraperitoneally depending on the proposed use. Generally, at least two groups of animals are

20 used in the assay, with at least one group being a control group that is administered the administration vehicle without the potential drug.

TABLE 2: TABLE OF SEQUENCES

SEQ ID NO:	Type	Description
1	N.A.	WT Hdm2 (17-125)
2	A.A.	WT Hdm2(17-125)
3	N.A.	modified Hdm2 (X ₁ -X ₇)
4	A.A.	modified Hdm2 (X ₁ -X ₇)
5	N.A.	modified Hdm2(Y76H)
6	A.A.	modified Hdm2(Y76H)
7	N.A.	modified Hdm2(F55Y)
8	A.A.	modified Hdm2(F55Y)
9	N.A.	modified Hdm2 (F55Y/Y76H)
10	A.A.	modified Hdm2 (F55Y/Y76H)
11	N.A.	modified Hdm2 (HK ₅)
12	A.A.	modified Hdm2 (HK ₅)

<u>SEQ ID NO:</u>	<u>Type</u>	<u>Description</u>
13	N.A.	Hdm2(F55Y) Primer
14	N.A.	RChdm2(F55Y) Primer
15	N.A.	Hdm2(Y76H) Primer
16	N.A.	RChdm2(Y76H)
17	N.A.	Y104S-GTAILOR-F Primer
18	N.A.	Y104-GTAILOR-R Primer

The present invention may be better understood by reference to the following non-limiting examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate
 5 certain embodiments of the invention. They should in no way be construed as limiting the broad scope of the invention.

EXAMPLES

EXAMPLE 1: Preparation of Modified Hdm2

Preparation of Hdm2 Constructs

10 Hdm2 was either modified with a single amino acid change, or with a double amino acid change using the QuickChange kit (Stratagene, La Jolla, CA, USA) and the pET32-Xa/LIC-hdm2 (17-125) vector as a template. The pET32-Xa/LIC parental vector was obtained from Novagen (San Diego, CA). Hdm2(F55Y), Hdm2(Y76H) and Hdm2(F55Y/Y76H) were constructed in this
 15 manner.

A modified Hdm2 having three amino acid substitutions, Hdm2(F55Y/Y76H/Y104S) was generated using GENETAILOR Site-directed Mutagenesis system (Invitrogen, Carlsbad, CA, USA) with the vector indicated above as the template. The following primers were used to generate the above-
 20 identified modified Hdm2 proteins:

(1) Hdm2(F55Y) Primer :SEQ ID NO:13

5' CTATGAAAGAGGTTCTTATTATCTTGGCCAGTATATTATGAC 3'

(2) RChdm2(F55Y) Primer: SEQ ID NO:14

5' GTCATAATATACTGGCCAAGATAATAAAGAACCTTTCATAG 3'

(3) Hdm2(Y76H) Primer: SEQ ID NO:15
5' GAGAAGCAACAAACATATTGTACATTGTTCAAATGATCTTCTAGG 3'
(4) RChdm2(Y76H) Primer: SEQ ID NO:16
5' CCTAGAACATTCATTGAACAAATGTACAATATGTTGTTGCTTCTC 3'
5 (5) Y104S-GTAILOR-F Primer: SEQ ID NO:17
5' CAGGAACCTGGTAGTAGTCAATCAGCAGG 3'
(6) Y104-GTAILOR-R Primer: SEQ ID NO:18
5' GACTACTACCAAGTTCCCTGGAGATCATGGT 3'

QuickChange mutagenesis was performed in two steps as previously described [Wang *et al.*, *BioTechniques* 26:680-682(1999)]. In the first stage two extension reactions were performed in separate tubes; one containing the forward primer and the other containing the reverse primer. After two cycles, the two reactions were mixed and the standard QuickChange mutagenesis procedure was carried out for an additional 18 cycles. Following amplification, the parental strand was digested with 1U of *Dpn*1 for 2 hours and an aliquot was transformed into DH5- α cells. The GENETAILOR mutagenesis was performed according to the manufacturer's instructions except that *pfu* turbo DNA polymerase was used instead of the polymerase recommended by manufacturer. All constructs were confirmed by sequencing (GeneWiz, New York, NY).

20 *Expression of modified Hdm2*

A colony from freshly transformed cells was grown at 37 °C to an optical density (OD) of 2.0 in 10ml TERRIFIC broth (Mediatech, Inc.) containing 100 µg/ml carbenicillin and 1% glucose. This 10ml culture was then used to inoculate a 1 liter culture having the same medium composition. The 1 liter culture was 25 grown at 37 °C to an OD of 2.0, stored at 4 °C overnight, and then used to inoculate a 10 liter tank containing TERRIFIC broth and 100µg/ml carbenicillin. The 10 liter culture was grown at 37 °C to an OD of 1.5-2.0 before lowering the temperature to 16 °C. The 10 liter culture was then induced with 1mM IPTG, and the cells were harvested 18 hours post-induction.

Purification of modified Hdm-2 proteins

The purification protocol as exemplified herein for Hdm2(F55Y/Y76H) is applicable for all of the modified Hdm2 proteins of the present invention.

IPTG-induced cells containing Hdm2(F55Y/Y76H) were harvested from
5 the 10 liter fermentation, as described above. The cells were suspended in 500 ml of 50 mM Tris-Cl Buffer, pH 8.0_{rt} (room temperature), 0.3 M NaCl, 10% (v/v) glycerol, 5 mM β-mercaptoethanol, 25 mM imidazole, 18,000 Units/liter endonuclease (ultrapure benzonase; SIGMA), and 6 ml/liter of CALBIOCHEM Protease Inhibitor Cocktail III. (All processing was performed at 4 °C.) To
10 homogenize the resulting cell suspension, it was passed through a large OMNI Mixer probe for 45 seconds, three times. The cell suspension was kept on ice for 2 minutes between each 45 second passage. The cells were then broken by three passages of the homogenized cell suspension through a Microfluidizer. The extract was recovered by centrifugation at 205,000 X g for 80 minutes at 4°
15 C.

The resulting 645 ml extract was mixed end over end for 50 minutes with 28 ml of QIAGEN Ni-NTA SUPERFLOW resin, which had been equilibrated with the equilibration buffer [50 mM Tris-Cl, pH 8.0_{rt}, 0.3 M NaCl, 5 mM β-mercaptoethanol and 25 mM imidazole]. The supernatant was decanted, and the
20 resin was then washed with 600 ml of the equilibration buffer. The resin was poured into a 2.6 x 5.3 cm column, washed with an additional 200 ml of equilibration buffer at 3.6 ml/min, and finally eluted with 50 mM Tris-Cl, pH 8.0_{rt}, 0.1 M NaCl, 250 mM imidazole, 5 mM β-mercaptoethanol and 20% glycerol.

A 0.64 ml volume of 0.5 M CaCl₂ was added to the eluted fusion protein
25 pool (195 mg of protein in 63 ml of elution buffer). The pooled protein was then diluted to 1 mg/ml with 50 mM Tris-Cl, pH 8.0_{rt}, 0.1 M NaCl, 10% glycerol, 5 mM CaCl₂ and 5 mM β-mercaptoethanol. A 1.95 ml volume of 2000 Units/ml Factor Xa protease (NOVAGEN) was added, and the pooled protein was dialyzed overnight versus 3.87 liters of the same buffer.

30 A 4.33 ml volume of 1M imidazole, pH 8.0, was added to the 200 ml of cleaved, pooled fusion protein, to bring the imidazole concentration to 24 mM.

The pooled protein was then applied at a rate of 3.6 ml/min to a 50 ml (2.6 x 9.4 cm) column of QIAGEN Ni-NTA SUPERFLOW resin that had been equilibrated with equilibration buffer. The column was then washed with the equilibration buffer.

5 The 230 ml flow-through was dialyzed versus three changes (6 liters, 5 liters and 5 liters) of Buffer A [25 mM Hepes-KOH, pH 7.5, 0.15 M KCl, 1 mM Na₂-EDTA, 0.03% sodium azide and 5 mM dithiothreitol]. All manipulations of the modified Hdm2 were in Buffer A from this point on. The dialyzed pooled protein was concentrated to 8.5 ml with an AMICON YM10 membrane,

10 centrifuged at 205,000 X g for 15 minutes, and then applied to a 2.6x 60 cm column of PHARMACIA SUPERDEX-75 at a flow rate of 0.8 ml/min. The resulting eluant was collected in 3.2 ml fractions.

Fractions 66-75 contained the purified, modified Hdm2 monomer. These fractions were pooled and a protein concentration of 3.6 mg/ml was determined
15 using $\epsilon_{276} = 10,150 \text{ M}^{-1} \text{ cm}^{-1}$ in 20 mM sodium phosphate, pH 6.5, with 6 M guanidine hydrochloride (ExPASy-ProtParam Tool). This determination correlated with that determined by the Bradford dye binding assay (BIORAD), using bovine serum albumin as the protein standard.

EXAMPLE 2: Preparation and crystallization of Hdm2 F55Y/Y76H-Tripeptide
20 **Complex**

The modified Hdm2 (F55Y/Y76H) protein was prepared by means of QuickChange mutagenesis as disclosed in Example 1 using the appropriate primers. A small scale expression study was carried out to evaluate the solubility and expression level of this construct. The expression level of the
25 soluble Hdm2(F55Y/Y76H) protein is 2 to 3 fold higher than wild type. 110 mgs of the Hdm2(F55Y/Y76H) was purified from a 10L culture through the four-step purification protocol described above.

A 13 ml aliquot of the 3.6 mg/ml pool was concentrated to a 1.25 mM concentration (in 3ml), on an AMICON 5000 mwco Ultrafree membrane. A 3 ml
30 aliquot of 8.5 mM SCH549128 (MW= 535) in Buffer A was added to the

concentrated aliquot. The complex was incubated at room temperature for 10 minutes, and then concentrated as above, to a final volume of 1.3 ml.

The HDM2(F55Y/Y76H) protein-tripeptide complex was crystallized using a hanging-drop vapor diffusion method. The protein (0.5 μ l; 34 mg/ml) in 25 mM Hepes-potassium hydroxide, pH7.5, 0.15 M potassium chloride, 1 mM EDTA, 0.03% sodium azide and 5 mM DTT buffer was mixed with an equal volume of precipitant solution [1.4 M tri sodium citrate, 0.1 M sodium Hepes, pH 7.5] placed on the underside of a siliconized glass coverslip and sealed in close proximity to 1 ml of the precipitant solution. Crystallization plates were incubated at 22 °C; rectangular rod crystals (0.02 x 0.2 mm) grew over 2-30 days.

Prior to data collection, crystals were washed with the reservoir solution of the crystallization setup and transferred into the same solution with 20% glycerol added. The crystals were then flash-cooled in a nitrogen stream at 95 K. X-ray diffraction was collected using a Rigaku generator equipped with a Raxis 4++ detector. Data were integrated and scaled using the HKL package.

<u>Data collection statistics:</u>	
Resolution	50.0 – 1.70 Å
No. of collected reflections	135908
No. of unique reflections($F \geq 0$)	12484
R-sym	6.7 %
Percent of theoretical ($I/s \geq 1$)	98.7%
Unit Cell	$a=37.999 \text{ Å}$, $b=45.333 \text{ Å}$, $c=63.999 \text{ Å}$, $\alpha=\beta=\gamma=90^\circ$
Space Group	P2 ₁ 2 ₁ 2 ₁
Asymmetric unit	1 molecule

The crystal structure was solved using molecular replacement using the search models 1YCQ and 1YCR from the PDB. Refinement was done using the program CNX.

Theoretical number of reflections 9121

	Resolution Limits	50.0 – 1.90 Å
	Number of unobserved reflections	106 (1.2%)
	Number of reflections in working set	8592 (98.8%)
5	Number of reflections in test set	423 (4.6%)
	Number of protein residues	87
	Number of solvent atoms	44
	R-factor	0.223
	R-free	0.243
10	RMSD bond length	0.0083 Å
	RMSD bond angles	1.46°

The structural coordinates for the above-described Hdm2 crystal are set forth below in Table 3, which is in Protein Data Bank (PDB) file format. The
15 numbered columns refer to the following:

	<u>Col. #</u>	<u>Reference</u>
	1	Atomic coordinate records for standard groups
	2	Atom serial number
	3	Atom name
20	4	Residue name
	5	Residue sequence number
	6	Orthogonal coordinates for X in Angstroms
	7	Orthogonal coordinates for Y in Angstroms
	8	Orthogonal coordinates for Z in Angstroms
25	9	Occupancy
	10	Temperature factor
	11	Element symbol

TABLE 3

1	2	3	4	5	6	7	8	9	10	11
ATOM	1	CB	GLU	25	8.599	29.031	2.725	1.00	52.10	C
ATOM	2	CG	GLU	25	7.336	29.192	3.556	1.00	55.02	C
ATOM	3	CD	GLU	25	6.648	27.844	3.697	1.00	57.01	C
ATOM	4	OE1	GLU	25	5.517	27.697	3.186	1.00	58.12	O
ATOM	5	OE2	GLU	25	7.235	26.932	4.322	1.00	57.62	O
ATOM	6	C	GLU	25	10.683	30.035	1.753	1.00	47.48	C
ATOM	7	O	GLU	25	10.654	29.682	0.574	1.00	48.97	O
ATOM	8	N	GLU	25	8.564	31.353	1.814	1.00	50.30	N
ATOM	9	CA	GLU	25	9.389	30.323	2.511	1.00	49.61	C
ATOM	10	N	THR	26	11.816	30.195	2.431	1.00	43.07	N
ATOM	11	CA	THR	26	13.119	29.941	1.824	1.00	37.71	C
ATOM	12	CB	THR	26	14.267	30.244	2.807	1.00	38.14	C
ATOM	13	OG1	THR	26	14.227	31.628	3.174	1.00	38.51	O
ATOM	14	CG2	THR	26	15.613	29.942	2.163	1.00	37.88	C
ATOM	15	C	THR	26	13.216	28.482	1.385	1.00	34.76	C
ATOM	16	O	THR	26	12.823	27.579	2.120	1.00	34.28	O
ATOM	17	N	LEU	27	13.730	28.261	0.179	1.00	29.30	N
ATOM	18	CA	LEU	27	13.872	26.915	-0.365	1.00	28.75	C
ATOM	19	CB	LEU	27	13.479	26.866	-1.842	1.00	28.72	C
ATOM	20	CG	LEU	27	12.043	27.255	-2.190	1.00	29.98	C
ATOM	21	CD1	LEU	27	11.823	27.208	-3.696	1.00	30.85	C
ATOM	22	CD2	LEU	27	11.037	26.358	-1.482	1.00	30.07	C
ATOM	23	C	LEU	27	15.240	26.271	-0.161	1.00	26.20	C
ATOM	24	O	LEU	27	16.270	26.922	-0.316	1.00	26.92	O
ATOM	25	N	VAL	28	15.234	24.989	0.196	1.00	24.29	N
ATOM	26	CA	VAL	28	16.465	24.230	0.402	1.00	22.04	C
ATOM	27	CB	VAL	28	16.566	23.684	1.845	1.00	21.66	C
ATOM	28	CG1	VAL	28	16.716	24.839	2.822	1.00	20.57	C
ATOM	29	CG2	VAL	28	15.337	22.849	2.180	1.00	18.74	C
ATOM	30	C	VAL	28	16.549	23.066	-0.585	1.00	22.51	C
ATOM	31	O	VAL	28	15.526	22.500	-0.979	1.00	20.60	O
ATOM	32	N	ARG	29	17.770	22.733	-0.996	1.00	21.42	N
ATOM	33	CA	ARG	29	18.010	21.641	-1.937	1.00	23.64	C
ATOM	34	CB	ARG	29	18.869	22.116	-3.107	1.00	27.62	C
ATOM	35	CG	ARG	29	19.162	21.053	-4.151	1.00	33.83	C
ATOM	36	CD	ARG	29	20.043	21.611	-5.267	1.00	38.61	C
ATOM	37	NE	ARG	29	19.435	22.740	-5.965	1.00	42.13	N
ATOM	38	CZ	ARG	29	18.360	22.653	-6.742	1.00	45.54	C
ATOM	39	NH1	ARG	29	17.763	21.483	-6.929	1.00	47.02	N
ATOM	40	NH2	ARG	29	17.882	23.740	-7.337	1.00	46.93	N
ATOM	41	C	ARG	29	18.696	20.470	-1.227	1.00	20.05	C
ATOM	42	O	ARG	29	19.851	20.573	-0.819	1.00	18.84	O
ATOM	43	N	PRO	30	17.988	19.343	-1.078	1.00	19.58	N
ATOM	44	CD	PRO	30	16.511	19.312	-1.031	1.00	21.57	C
ATOM	45	CA	PRO	30	18.498	18.137	-0.425	1.00	20.02	C
ATOM	46	CB	PRO	30	17.278	17.234	-0.444	1.00	22.34	C
ATOM	47	CG	PRO	30	16.234	18.184	0.016	1.00	21.93	C
ATOM	48	C	PRO	30	19.678	17.493	-1.160	1.00	19.19	C
ATOM	49	O	PRO	30	19.624	17.323	-2.379	1.00	18.14	O
ATOM	50	N	LYS	31	20.737	17.144	-0.433	1.00	17.57	N
ATOM	51	CA	LYS	31	21.870	16.450	-1.044	1.00	17.54	C
ATOM	52	CB	LYS	31	23.083	16.416	-0.110	1.00	17.17	C
ATOM	53	CG	LYS	31	23.588	17.814	0.242	1.00	21.31	C
ATOM	54	CD	LYS	31	24.792	17.784	1.175	1.00	24.34	C
ATOM	55	CE	LYS	31	25.250	19.198	1.499	1.00	27.70	C

ATOM	56	NZ	LYS	31	26.424	19.216	2.411	1.00	30.99	N
ATOM	57	C	LYS	31	21.367	15.049	-1.436	1.00	18.76	C
ATOM	58	O	LYS	31	20.316	14.612	-0.961	1.00	17.24	O
ATOM	59	N	PRO	32	22.093	14.338	-2.319	1.00	18.30	N
ATOM	60	CD	PRO	32	23.329	14.791	-2.991	1.00	21.43	C
ATOM	61	CA	PRO	32	21.764	13.000	-2.820	1.00	19.62	C
ATOM	62	CB	PRO	32	23.111	12.503	-3.293	1.00	22.05	C
ATOM	63	CG	PRO	32	23.536	13.680	-4.080	1.00	21.40	C
ATOM	64	C	PRO	32	21.004	11.990	-1.947	1.00	19.34	C
ATOM	65	O	PRO	32	19.883	11.591	-2.299	1.00	17.19	O
ATOM	66	N	LEU	33	21.594	11.559	-0.833	1.00	18.45	N
ATOM	67	CA	LEU	33	20.911	10.581	0.017	1.00	18.88	C
ATOM	68	CB	LEU	33	21.788	10.020	1.142	1.00	21.73	C
ATOM	69	CG	LEU	33	23.050	9.209	0.871	1.00	28.04	C
ATOM	70	CD1	LEU	33	24.067	9.989	0.052	1.00	29.17	C
ATOM	71	CD2	LEU	33	23.663	8.738	2.191	1.00	29.05	C
ATOM	72	C	LEU	33	19.597	11.062	0.602	1.00	17.92	C
ATOM	73	O	LEU	33	18.603	10.340	0.565	1.00	16.48	O
ATOM	74	N	LEU	34	19.588	12.277	1.140	1.00	17.19	N
ATOM	75	CA	LEU	34	18.365	12.817	1.719	1.00	15.57	C
ATOM	76	CB	LEU	34	18.632	14.142	2.431	1.00	17.51	C
ATOM	77	CG	LEU	34	17.436	14.863	3.052	1.00	18.19	C
ATOM	78	CD1	LEU	34	16.656	13.966	4.007	1.00	16.62	C
ATOM	79	CD2	LEU	34	17.885	16.147	3.738	1.00	16.73	C
ATOM	80	C	LEU	34	17.316	12.995	0.638	1.00	17.16	C
ATOM	81	O	LEU	34	16.128	12.769	0.870	1.00	16.85	O
ATOM	82	N	LEU	35	17.755	13.385	-0.553	1.00	15.69	N
ATOM	83	CA	LEU	35	16.814	13.578	-1.644	1.00	18.62	C
ATOM	84	CB	LEU	35	17.508	14.148	-2.885	1.00	20.48	C
ATOM	85	CG	LEU	35	16.605	14.406	-4.097	1.00	23.23	C
ATOM	86	CD1	LEU	35	15.470	15.356	-3.733	1.00	23.45	C
ATOM	87	CD2	LEU	35	17.389	14.944	-5.293	1.00	23.24	C
ATOM	88	C	LEU	35	16.160	12.238	-1.966	1.00	19.55	C
ATOM	89	O	LEU	35	14.951	12.155	-2.195	1.00	17.74	O
ATOM	90	N	LYS	36	16.964	11.181	-1.955	1.00	18.27	N
ATOM	91	CA	LYS	36	16.453	9.859	-2.271	1.00	22.37	C
ATOM	92	CB	LYS	36	17.610	8.864	-2.413	1.00	24.10	C
ATOM	93	CG	LYS	36	17.212	7.444	-2.785	1.00	30.65	C
ATOM	94	CD	LYS	36	18.461	6.576	-2.920	1.00	32.62	C
ATOM	95	CE	LYS	36	18.128	5.145	-3.307	1.00	36.89	C
ATOM	96	NZ	LYS	36	19.362	4.312	-3.442	1.00	37.42	N
ATOM	97	C	LYS	36	15.413	9.413	-1.246	1.00	22.40	C
ATOM	98	O	LYS	36	14.435	8.751	-1.597	1.00	22.93	O
ATOM	99	N	LEU	37	15.605	9.802	0.013	1.00	22.25	N
ATOM	100	CA	LEU	37	14.659	9.432	1.065	1.00	21.53	C
ATOM	101	CB	LEU	37	15.231	9.702	2.453	1.00	24.79	C
ATOM	102	CG	LEU	37	16.511	8.980	2.865	1.00	27.14	C
ATOM	103	CD1	LEU	37	16.996	9.454	4.226	1.00	29.28	C
ATOM	104	CD2	LEU	37	16.317	7.474	2.855	1.00	29.24	C
ATOM	105	C	LEU	37	13.355	10.197	0.894	1.00	22.46	C
ATOM	106	O	LEU	37	12.269	9.636	1.053	1.00	19.80	O
ATOM	107	N	LEU	38	13.470	11.479	0.557	1.00	21.33	N
ATOM	108	CA	LEU	38	12.296	12.322	0.369	1.00	21.81	C
ATOM	109	CB	LEU	38	12.693	13.794	0.252	1.00	22.60	C
ATOM	110	CG	LEU	38	13.394	14.398	1.467	1.00	23.43	C
ATOM	111	CD1	LEU	38	13.905	15.806	1.185	1.00	24.29	C
ATOM	112	CD2	LEU	38	12.491	14.362	2.685	1.00	24.94	C
ATOM	113	C	LEU	38	11.483	11.884	-0.836	1.00	24.00	C
ATOM	114	O	LEU	38	10.254	11.986	-0.830	1.00	25.09	O

ATOM	115	N	LYS	39	12.160	11.381	-1.865	1.00	24.12	N
ATOM	116	CA	LYS	39	11.442	10.928	-3.045	1.00	28.66	C
ATOM	117	CB	LYS	39	12.339	10.855	-4.282	1.00	29.59	C
ATOM	118	CG	LYS	39	12.939	12.179	-4.728	1.00	30.58	C
ATOM	119	CD	LYS	39	13.798	11.969	-5.968	1.00	34.21	C
ATOM	120	CE	LYS	39	14.414	13.266	-6.459	1.00	35.99	C
ATOM	121	NZ	LYS	39	13.376	14.268	-6.818	1.00	37.93	N
ATOM	122	C	LYS	39	10.766	9.588	-2.803	1.00	29.02	C
ATOM	123	O	LYS	39	9.784	9.256	-3.468	1.00	30.57	O
ATOM	124	N	SER	40	11.270	8.823	-1.839	1.00	27.39	N
ATOM	125	CA	SER	40	10.671	7.526	-1.557	1.00	27.21	C
ATOM	126	CB	SER	40	11.619	6.625	-0.768	1.00	29.11	C
ATOM	127	OG	SER	40	11.934	7.200	0.484	1.00	31.61	O
ATOM	128	C	SER	40	9.371	7.739	-0.791	1.00	26.87	C
ATOM	129	O	SER	40	8.522	6.850	-0.731	1.00	24.88	O
ATOM	130	N	VAL	41	9.217	8.931	-0.222	1.00	23.16	N
ATOM	131	CA	VAL	41	8.020	9.265	0.534	1.00	24.52	C
ATOM	132	CB	VAL	41	8.321	10.392	1.553	1.00	25.80	C
ATOM	133	CG1	VAL	41	7.053	10.879	2.197	1.00	27.99	C
ATOM	134	CG2	VAL	41	9.271	9.873	2.620	1.00	25.81	C
ATOM	135	C	VAL	41	6.933	9.718	-0.436	1.00	24.07	C
ATOM	136	O	VAL	41	5.750	9.780	-0.083	1.00	22.91	O
ATOM	137	N	GLY	42	7.343	10.018	-1.665	1.00	23.67	N
ATOM	138	CA	GLY	42	6.394	10.446	-2.678	1.00	23.84	C
ATOM	139	C	GLY	42	6.685	11.764	-3.373	1.00	22.51	C
ATOM	140	O	GLY	42	6.165	12.010	-4.461	1.00	21.84	O
ATOM	141	N	ALA	43	7.508	12.616	-2.767	1.00	22.69	N
ATOM	142	CA	ALA	43	7.817	13.916	-3.361	1.00	23.42	C
ATOM	143	CB	ALA	43	8.331	14.868	-2.287	1.00	26.17	C
ATOM	144	C	ALA	43	8.830	13.811	-4.497	1.00	23.99	C
ATOM	145	O	ALA	43	9.996	13.496	-4.270	1.00	23.65	O
ATOM	146	N	GLN	44	8.369	14.091	-5.715	1.00	23.66	N
ATOM	147	CA	GLN	44	9.194	14.028	-6.925	1.00	26.00	C
ATOM	148	CB	GLN	44	8.416	13.405	-8.091	1.00	27.49	C
ATOM	149	CG	GLN	44	7.957	11.970	-7.853	1.00	29.74	C
ATOM	150	CD	GLN	44	9.160	11.066	-7.633	1.00	33.73	C
ATOM	151	OE1	GLN	44	10.295	11.436	-7.932	1.00	36.21	O
ATOM	152	NE2	GLN	44	8.915	9.878	-7.092	1.00	35.39	N
ATOM	153	C	GLN	44	9.814	15.363	-7.344	1.00	28.84	C
ATOM	154	O	GLN	44	9.895	15.669	-8.532	1.00	33.20	O
ATOM	155	N	LYS	45	10.239	16.162	-6.373	1.00	26.37	N
ATOM	156	CA	LYS	45	10.849	17.453	-6.676	1.00	26.53	C
ATOM	157	CB	LYS	45	9.937	18.606	-6.267	1.00	26.39	C
ATOM	158	CG	LYS	45	9.609	18.638	-4.794	1.00	26.84	C
ATOM	159	CD	LYS	45	8.700	19.806	-4.469	1.00	28.76	C
ATOM	160	CE	LYS	45	8.370	19.843	-2.986	1.00	27.65	C
ATOM	161	NZ	LYS	45	7.477	20.988	-2.669	1.00	30.55	N
ATOM	162	C	LYS	45	12.229	17.574	-6.037	1.00	25.49	C
ATOM	163	O	LYS	45	12.575	16.786	-5.161	1.00	24.24	O
ATOM	164	N	ASP	46	13.020	18.548	-6.480	1.00	26.71	N
ATOM	165	CA	ASP	46	14.360	18.726	-5.925	1.00	28.66	C
ATOM	166	CB	ASP	46	15.403	18.827	-7.040	1.00	35.21	C
ATOM	167	CG	ASP	46	15.144	19.991	-7.980	1.00	40.54	C
ATOM	168	OD1	ASP	46	14.152	20.724	-7.771	1.00	42.44	O
ATOM	169	OD2	ASP	46	15.936	20.172	-8.932	1.00	45.73	O
ATOM	170	C	ASP	46	14.534	19.865	-4.913	1.00	25.87	C
ATOM	171	O	ASP	46	15.587	19.975	-4.286	1.00	24.85	O
ATOM	172	N	THR	47	13.513	20.703	-4.747	1.00	23.77	N
ATOM	173	CA	THR	47	13.586	21.811	-3.788	1.00	23.33	C

ATOM	174	CB	THR	47	13.618	23.193	-4.476	1.00	25.02	C
ATOM	175	OG1	THR	47	12.413	23.394	-5.219	1.00	27.77	O
ATOM	176	CG2	THR	47	14.814	23.291	-5.398	1.00	26.67	C
ATOM	177	C	THR	47	12.398	21.764	-2.843	1.00	21.70	C
ATOM	178	O	THR	47	11.266	21.522	-3.263	1.00	21.88	O
ATOM	179	N	TYR	48	12.670	21.995	-1.563	1.00	19.26	N
ATOM	180	CA	TYR	48	11.649	21.927	-0.524	1.00	19.39	C
ATOM	181	CB	TYR	48	11.819	20.647	0.286	1.00	20.20	C
ATOM	182	CG	TYR	48	11.751	19.375	-0.517	1.00	20.69	C
ATOM	183	CD1	TYR	48	10.643	18.539	-0.433	1.00	20.32	C
ATOM	184	CE1	TYR	48	10.577	17.366	-1.170	1.00	23.35	C
ATOM	185	CD2	TYR	48	12.799	19.007	-1.362	1.00	20.03	C
ATOM	186	CE2	TYR	48	12.744	17.836	-2.107	1.00	22.54	C
ATOM	187	CZ	TYR	48	11.631	17.021	-2.007	1.00	21.66	C
ATOM	188	OH	TYR	48	11.564	15.867	-2.747	1.00	24.12	O
ATOM	189	C	TYR	48	11.761	23.093	0.444	1.00	18.48	C
ATOM	190	O	TYR	48	12.732	23.838	0.420	1.00	18.96	O
ATOM	191	N	THR	49	10.747	23.246	1.286	1.00	18.95	N
ATOM	192	CA	THR	49	10.792	24.247	2.344	1.00	19.64	C
ATOM	193	CB	THR	49	9.398	24.779	2.729	1.00	20.36	C
ATOM	194	OG1	THR	49	8.595	23.704	3.229	1.00	18.68	O
ATOM	195	CG2	THR	49	8.720	25.421	1.531	1.00	21.70	C
ATOM	196	C	THR	49	11.332	23.403	3.503	1.00	19.26	C
ATOM	197	O	THR	49	11.187	22.177	3.491	1.00	16.72	O
ATOM	198	N	MET	50	11.971	24.033	4.482	1.00	19.49	N
ATOM	199	CA	MET	50	12.496	23.287	5.621	1.00	19.92	C
ATOM	200	CB	MET	50	13.164	24.227	6.623	1.00	19.27	C
ATOM	201	CG	MET	50	13.752	23.549	7.857	1.00	21.93	C
ATOM	202	SD	MET	50	15.038	22.346	7.482	1.00	22.54	S
ATOM	203	CE	MET	50	16.405	23.430	7.220	1.00	21.56	C
ATOM	204	C	MET	50	11.366	22.505	6.292	1.00	20.51	C
ATOM	205	O	MET	50	11.556	21.358	6.717	1.00	19.62	O
ATOM	206	N	LYS	51	10.184	23.113	6.370	1.00	20.47	N
ATOM	207	CA	LYS	51	9.055	22.446	7.008	1.00	21.99	C
ATOM	208	CB	LYS	51	7.860	23.388	7.165	1.00	26.64	C
ATOM	209	CG	LYS	51	6.659	22.735	7.834	1.00	33.29	C
ATOM	210	CD	LYS	51	5.483	23.695	7.983	1.00	38.26	C
ATOM	211	CE	LYS	51	4.301	23.005	8.660	1.00	40.81	C
ATOM	212	NZ	LYS	51	3.128	23.912	8.828	1.00	42.40	N
ATOM	213	C	LYS	51	8.650	21.172	6.273	1.00	19.90	C
ATOM	214	O	LYS	51	8.205	20.210	6.898	1.00	19.38	O
ATOM	215	N	GLU	52	8.817	21.149	4.953	1.00	18.87	N
ATOM	216	CA	GLU	52	8.464	19.944	4.205	1.00	17.93	C
ATOM	217	CB	GLU	52	8.382	20.189	2.699	1.00	18.18	C
ATOM	218	CG	GLU	52	7.344	21.203	2.247	1.00	23.24	C
ATOM	219	CD	GLU	52	7.396	21.313	0.732	1.00	25.60	C
ATOM	220	OE1	GLU	52	8.396	21.846	0.213	1.00	23.72	O
ATOM	221	OE2	GLU	52	6.438	20.874	0.057	1.00	26.89	O
ATOM	222	C	GLU	52	9.486	18.855	4.482	1.00	15.85	C
ATOM	223	O	GLU	52	9.135	17.685	4.619	1.00	16.69	O
ATOM	224	N	VAL	53	10.753	19.250	4.568	1.00	16.93	N
ATOM	225	CA	VAL	53	11.825	18.293	4.825	1.00	17.04	C
ATOM	226	CB	VAL	53	13.203	18.995	4.855	1.00	16.03	C
ATOM	227	CG1	VAL	53	14.280	18.019	5.304	1.00	16.51	C
ATOM	228	CG2	VAL	53	13.534	19.547	3.467	1.00	17.18	C
ATOM	229	C	VAL	53	11.596	17.602	6.166	1.00	15.46	C
ATOM	230	O	VAL	53	11.612	16.371	6.254	1.00	15.66	O
ATOM	231	N	LEU	54	11.368	18.401	7.203	1.00	15.11	N
ATOM	232	CA	LEU	54	11.136	17.868	8.539	1.00	18.02	C

ATOM	233	CB	LEU	54	11.038	19.004	9.564	1.00	18.06	C
ATOM	234	CG	LEU	54	12.275	19.897	9.715	1.00	19.94	C
ATOM	235	CD1	LEU	54	12.017	21.075	10.651	1.00	21.06	C
ATOM	236	CD2	LEU	54	13.484	19.088	10.172	1.00	19.37	C
ATOM	237	C	LEU	54	9.887	16.989	8.551	1.00	17.44	C
ATOM	238	O	LEU	54	9.890	15.901	9.132	1.00	17.96	O
ATOM	239	N	TYR	55	8.824	17.456	7.900	1.00	19.29	N
ATOM	240	CA	TYR	55	7.582	16.686	7.826	1.00	19.06	C
ATOM	241	CB	TYR	55	6.476	17.462	7.107	1.00	21.67	C
ATOM	242	CG	TYR	55	5.208	16.659	6.914	1.00	24.34	C
ATOM	243	CD1	TYR	55	4.331	16.414	7.970	1.00	27.75	C
ATOM	244	CE1	TYR	55	3.185	15.626	7.788	1.00	28.11	C
ATOM	245	CD2	TYR	55	4.910	16.100	5.671	1.00	26.71	C
ATOM	246	CE2	TYR	55	3.778	15.316	5.479	1.00	27.50	C
ATOM	247	CZ	TYR	55	2.920	15.082	6.537	1.00	29.01	C
ATOM	248	OH	TYR	55	1.802	14.302	6.332	1.00	31.62	O
ATOM	249	C	TYR	55	7.778	15.329	7.162	1.00	17.61	C
ATOM	250	O	TYR	55	7.450	14.295	7.742	1.00	18.75	O
ATOM	251	N	TYR	56	8.314	15.332	5.943	1.00	17.90	N
ATOM	252	CA	TYR	56	8.537	14.079	5.228	1.00	15.80	C
ATOM	253	CB	TYR	56	8.959	14.322	3.781	1.00	16.36	C
ATOM	254	CG	TYR	56	7.897	14.995	2.944	1.00	19.38	C
ATOM	255	CD1	TYR	56	6.589	14.508	2.934	1.00	18.67	C
ATOM	256	CE1	TYR	56	5.610	15.083	2.132	1.00	21.66	C
ATOM	257	CD2	TYR	56	8.201	16.083	2.125	1.00	19.49	C
ATOM	258	CE2	TYR	56	7.225	16.667	1.315	1.00	20.60	C
ATOM	259	CZ	TYR	56	5.935	16.160	1.325	1.00	22.09	C
ATOM	260	OH	TYR	56	4.967	16.724	0.524	1.00	24.00	O
ATOM	261	C	TYR	56	9.463	13.067	5.893	1.00	16.42	C
ATOM	262	O	TYR	56	9.247	11.863	5.759	1.00	16.20	O
ATOM	263	N	LEU	57	10.484	13.531	6.609	1.00	17.70	N
ATOM	264	CA	LEU	57	11.362	12.579	7.283	1.00	18.25	C
ATOM	265	CB	LEU	57	12.629	13.232	7.836	1.00	22.32	C
ATOM	266	CG	LEU	57	13.582	13.831	6.810	1.00	26.55	C
ATOM	267	CD1	LEU	57	14.757	14.543	7.473	1.00	27.16	C
ATOM	268	CD2	LEU	57	14.073	12.746	5.861	1.00	29.77	C
ATOM	269	C	LEU	57	10.588	11.891	8.385	1.00	18.46	C
ATOM	270	O	LEU	57	10.782	10.704	8.641	1.00	19.38	O
ATOM	271	N	GLY	58	9.693	12.639	9.023	1.00	16.71	N
ATOM	272	CA	GLY	58	8.883	12.060	10.077	1.00	18.09	C
ATOM	273	C	GLY	58	7.958	11.030	9.458	1.00	18.69	C
ATOM	274	O	GLY	58	7.750	9.948	10.012	1.00	17.12	O
ATOM	275	N	GLN	59	7.402	11.370	8.297	1.00	18.56	N
ATOM	276	CA	GLN	59	6.494	10.470	7.585	1.00	19.64	C
ATOM	277	CB	GLN	59	5.787	11.209	6.448	1.00	21.81	C
ATOM	278	CG	GLN	59	4.918	12.363	6.913	1.00	25.45	C
ATOM	279	CD	GLN	59	3.842	11.829	7.830	1.00	29.94	C
ATOM	280	OE1	GLN	59	3.753	12.211	9.000	1.00	32.36	O
ATOM	281	NE2	GLN	59	3.015	10.934	7.304	1.00	31.47	N
ATOM	282	C	GLN	59	7.212	9.228	7.062	1.00	18.28	C
ATOM	283	O	GLN	59	6.624	8.150	6.977	1.00	18.50	O
ATOM	284	N	TYR	60	8.488	9.391	6.725	1.00	17.72	N
ATOM	285	CA	TYR	60	9.322	8.294	6.237	1.00	19.36	C
ATOM	286	CB	TYR	60	10.699	8.816	5.829	1.00	19.10	C
ATOM	287	CG	TYR	60	11.675	7.746	5.386	1.00	21.10	C
ATOM	288	CD1	TYR	60	11.634	7.221	4.095	1.00	22.00	C
ATOM	289	CE1	TYR	60	12.526	6.224	3.691	1.00	20.83	C
ATOM	290	CD2	TYR	60	12.635	7.247	6.269	1.00	21.10	C
ATOM	291	CE2	TYR	60	13.532	6.245	5.876	1.00	20.35	C

ATOM	292	CZ	TYR	60	13.469	5.740	4.585	1.00	20.69	C
ATOM	293	OH	TYR	60	14.329	4.746	4.187	1.00	20.09	O
ATOM	294	C	TYR	60	9.474	7.246	7.335	1.00	19.51	C
ATOM	295	O	TYR	60	9.283	6.046	7.113	1.00	18.29	O
ATOM	296	N	ILE	61	9.812	7.722	8.527	1.00	17.99	N
ATOM	297	CA	ILE	61	10.012	6.851	9.675	1.00	19.08	C
ATOM	298	CB	ILE	61	10.521	7.687	10.878	1.00	20.08	C
ATOM	299	CG2	ILE	61	10.452	6.869	12.164	1.00	20.84	C
ATOM	300	CG1	ILE	61	11.945	8.179	10.586	1.00	18.04	C
ATOM	301	CD1	ILE	61	12.547	9.033	11.670	1.00	20.08	C
ATOM	302	C	ILE	61	8.709	6.155	10.049	1.00	20.09	C
ATOM	303	O	ILE	61	8.692	4.975	10.406	1.00	19.98	O
ATOM	304	N	MET	62	7.606	6.879	9.933	1.00	20.53	N
ATOM	305	CA	MET	62	6.330	6.303	10.301	1.00	25.34	C
ATOM	306	CB	MET	62	5.305	7.398	10.599	1.00	28.26	C
ATOM	307	CG	MET	62	3.943	6.874	11.011	1.00	34.36	C
ATOM	308	SD	MET	62	2.800	8.211	11.360	1.00	41.93	S
ATOM	309	CE	MET	62	2.064	8.447	9.727	1.00	41.20	C
ATOM	310	C	MET	62	5.800	5.313	9.277	1.00	25.27	C
ATOM	311	O	MET	62	5.281	4.257	9.648	1.00	24.70	O
ATOM	312	N	THR	63	5.951	5.635	7.997	1.00	25.57	N
ATOM	313	CA	THR	63	5.441	4.755	6.954	1.00	28.86	C
ATOM	314	CB	THR	63	5.393	5.479	5.599	1.00	29.41	C
ATOM	315	OG1	THR	63	4.566	6.641	5.722	1.00	33.31	O
ATOM	316	CG2	THR	63	4.809	4.574	4.526	1.00	28.44	C
ATOM	317	C	THR	63	6.247	3.473	6.810	1.00	27.27	C
ATOM	318	O	THR	63	5.711	2.449	6.383	1.00	27.48	O
ATOM	319	N	LYS	64	7.526	3.510	7.168	1.00	26.66	N
ATOM	320	CA	LYS	64	8.324	2.295	7.087	1.00	26.49	C
ATOM	321	CB	LYS	64	9.730	2.517	6.523	1.00	26.55	C
ATOM	322	CG	LYS	64	9.740	3.088	5.117	1.00	29.11	C
ATOM	323	CD	LYS	64	11.150	3.247	4.571	1.00	28.43	C
ATOM	324	CE	LYS	64	11.850	1.886	4.510	1.00	29.76	C
ATOM	325	NZ	LYS	64	13.237	1.959	3.969	1.00	29.61	N
ATOM	326	C	LYS	64	8.375	1.642	8.453	1.00	25.50	C
ATOM	327	O	LYS	64	9.026	0.617	8.639	1.00	26.13	O
ATOM	328	N	ARG	65	7.678	2.255	9.403	1.00	24.76	N
ATOM	329	CA	ARG	65	7.607	1.754	10.765	1.00	25.45	C
ATOM	330	CB	ARG	65	6.672	0.542	10.843	1.00	31.11	C
ATOM	331	CG	ARG	65	5.246	0.879	10.397	1.00	35.38	C
ATOM	332	CD	ARG	65	4.303	-0.309	10.470	1.00	41.28	C
ATOM	333	NE	ARG	65	2.954	0.053	10.034	1.00	46.30	N
ATOM	334	CZ	ARG	65	1.897	-0.754	10.104	1.00	48.44	C
ATOM	335	NH1	ARG	65	2.023	-1.978	10.598	1.00	49.25	N
ATOM	336	NH2	ARG	65	0.712	-0.338	9.674	1.00	48.82	N
ATOM	337	C	ARG	65	8.979	1.472	11.378	1.00	25.07	C
ATOM	338	O	ARG	65	9.193	0.425	11.990	1.00	23.46	O
ATOM	339	N	LEU	66	9.910	2.409	11.202	1.00	22.51	N
ATOM	340	CA	LEU	66	11.259	2.246	11.745	1.00	21.93	C
ATOM	341	CB	LEU	66	12.251	3.184	11.063	1.00	21.83	C
ATOM	342	CG	LEU	66	12.406	3.035	9.552	1.00	21.63	C
ATOM	343	CD1	LEU	66	13.391	4.062	9.015	1.00	20.95	C
ATOM	344	CD2	LEU	66	12.838	1.634	9.170	1.00	20.51	C
ATOM	345	C	LEU	66	11.322	2.436	13.259	1.00	22.70	C
ATOM	346	O	LEU	66	12.325	2.115	13.893	1.00	22.69	O
ATOM	347	N	TYR	67	10.246	2.962	13.833	1.00	23.65	N
ATOM	348	CA	TYR	67	10.174	3.180	15.273	1.00	23.29	C
ATOM	349	CB	TYR	67	9.091	4.197	15.622	1.00	24.97	C
ATOM	350	CG	TYR	67	7.703	3.781	15.182	1.00	25.97	C

ATOM	351	CD1	TYR	67	6.928	2.923	15.966	1.00	27.64	C
ATOM	352	CE1	TYR	67	5.656	2.522	15.554	1.00	27.94	C
ATOM	353	CD2	TYR	67	7.172	4.227	13.972	1.00	27.22	C
ATOM	354	CE2	TYR	67	5.905	3.830	13.549	1.00	28.73	C
ATOM	355	CZ	TYR	67	5.153	2.980	14.345	1.00	29.04	C
ATOM	356	OH	TYR	67	3.899	2.594	13.930	1.00	29.83	O
ATOM	357	C	TYR	67	9.917	1.838	15.951	1.00	24.96	C
ATOM	358	O	TYR	67	9.215	0.992	15.404	1.00	26.17	O
ATOM	359	N	ASP	68	10.500	1.629	17.124	1.00	25.95	N
ATOM	360	CA	ASP	68	10.282	0.376	17.835	1.00	28.28	C
ATOM	361	CB	ASP	68	11.205	0.225	19.038	1.00	31.87	C
ATOM	362	CG	ASP	68	10.969	-1.082	19.781	1.00	35.20	C
ATOM	363	OD1	ASP	68	10.683	-1.042	20.994	1.00	36.44	O
ATOM	364	OD2	ASP	68	11.061	-2.149	19.141	1.00	36.99	O
ATOM	365	C	ASP	68	8.827	0.260	18.262	1.00	28.80	C
ATOM	366	O	ASP	68	8.286	1.157	18.907	1.00	23.20	O
ATOM	367	N	GLU	69	8.200	-0.851	17.898	1.00	32.27	N
ATOM	368	CA	GLU	69	6.799	-1.073	18.224	1.00	36.70	C
ATOM	369	CB	GLU	69	6.336	-2.428	17.684	1.00	40.26	C
ATOM	370	CG	GLU	69	4.873	-2.762	17.936	1.00	44.82	C
ATOM	371	CD	GLU	69	3.981	-1.727	17.266	1.00	48.03	C
ATOM	372	OE1	GLU	69	3.189	-2.115	16.380	1.00	50.17	O
ATOM	373	OE2	GLU	69	4.064	-0.532	17.621	1.00	50.09	O
ATOM	374	C	GLU	69	6.490	-0.956	19.719	1.00	36.29	C
ATOM	375	O	GLU	69	5.376	-0.587	20.089	1.00	35.50	O
ATOM	376	N	LYS	70	7.471	-1.245	20.574	1.00	36.45	N
ATOM	377	CA	LYS	70	7.246	-1.173	22.018	1.00	37.74	C
ATOM	378	CB	LYS	70	7.591	-2.501	22.694	1.00	40.70	C
ATOM	379	CG	LYS	70	6.766	-3.677	22.186	1.00	43.93	C
ATOM	380	CD	LYS	70	7.137	-4.979	22.889	1.00	46.97	C
ATOM	381	CE	LYS	70	6.298	-6.143	22.369	1.00	47.54	C
ATOM	382	NZ	LYS	70	6.634	-7.434	23.042	1.00	48.98	N
ATOM	383	C	LYS	70	7.928	-0.005	22.730	1.00	37.66	C
ATOM	384	O	LYS	70	7.557	0.348	23.852	1.00	39.60	O
ATOM	385	N	GLN	71	8.918	0.595	22.080	1.00	33.78	N
ATOM	386	CA	GLN	71	9.615	1.749	22.643	1.00	31.65	C
ATOM	387	CB	GLN	71	11.073	1.425	22.967	1.00	33.12	C
ATOM	388	CG	GLN	71	11.259	0.266	23.920	1.00	35.60	C
ATOM	389	CD	GLN	71	12.739	0.054	24.162	1.00	36.23	C
ATOM	390	OE1	GLN	71	13.361	-0.811	23.548	1.00	35.70	O
ATOM	391	NE2	GLN	71	13.305	0.826	25.081	1.00	35.71	N
ATOM	392	C	GLN	71	9.530	2.706	21.466	1.00	29.31	C
ATOM	393	O	GLN	71	10.488	2.864	20.713	1.00	23.70	O
ATOM	394	N	GLN	72	8.378	3.349	21.322	1.00	27.87	N
ATOM	395	CA	GLN	72	8.142	4.223	20.185	1.00	29.09	C
ATOM	396	CB	GLN	72	6.650	4.530	20.041	1.00	32.14	C
ATOM	397	CG	GLN	72	5.800	3.283	19.830	1.00	35.48	C
ATOM	398	CD	GLN	72	4.345	3.680	19.685	1.00	37.97	C
ATOM	399	OE1	GLN	72	3.689	3.324	18.707	1.00	40.02	O
ATOM	400	NE2	GLN	72	3.836	4.431	20.654	1.00	39.88	N
ATOM	401	C	GLN	72	8.975	5.480	20.002	1.00	27.04	C
ATOM	402	O	GLN	72	8.862	6.134	18.968	1.00	28.04	O
ATOM	403	N	HIS	73	9.814	5.823	20.973	1.00	25.52	N
ATOM	404	CA	HIS	73	10.638	7.017	20.817	1.00	26.50	C
ATOM	405	CB	HIS	73	10.876	7.728	22.155	1.00	29.45	C
ATOM	406	CG	HIS	73	11.649	6.914	23.147	1.00	33.08	C
ATOM	407	CD2	HIS	73	11.240	6.122	24.167	1.00	34.44	C
ATOM	408	ND1	HIS	73	13.025	6.847	23.136	1.00	34.94	N
ATOM	409	CE1	HIS	73	13.432	6.048	24.109	1.00	35.16	C

ATOM	410	NE2	HIS	73	12.369	5.596	24.748	1.00	34.82	N
ATOM	411	C	HIS	73	11.968	6.628	20.170	1.00	25.41	C
ATOM	412	O	HIS	73	12.763	7.494	19.797	1.00	24.29	O
ATOM	413	N	ILE	74	12.200	5.322	20.031	1.00	22.09	N
ATOM	414	CA	ILE	74	13.439	4.827	19.433	1.00	19.61	C
ATOM	415	CB	ILE	74	13.967	3.575	20.159	1.00	19.95	C
ATOM	416	CG2	ILE	74	15.274	3.118	19.517	1.00	20.34	C
ATOM	417	CG1	ILE	74	14.185	3.888	21.640	1.00	15.63	C
ATOM	418	CD1	ILE	74	14.696	2.712	22.442	1.00	20.76	C
ATOM	419	C	ILE	74	13.275	4.472	17.966	1.00	20.12	C
ATOM	420	O	ILE	74	12.440	3.642	17.593	1.00	19.78	O
ATOM	421	N	VAL	75	14.083	5.107	17.131	1.00	18.20	N
ATOM	422	CA	VAL	75	14.034	4.856	15.703	1.00	17.89	C
ATOM	423	CB	VAL	75	14.193	6.171	14.901	1.00	17.74	C
ATOM	424	CG1	VAL	75	14.278	5.862	13.413	1.00	20.35	C
ATOM	425	CG2	VAL	75	13.025	7.106	15.185	1.00	19.50	C
ATOM	426	C	VAL	75	15.152	3.909	15.297	1.00	18.16	C
ATOM	427	O	VAL	75	16.326	4.218	15.493	1.00	18.79	O
ATOM	428	N	HIS	76	14.788	2.754	14.745	1.00	18.90	N
ATOM	429	CA	HIS	76	15.775	1.785	14.271	1.00	20.21	C
ATOM	430	CB	HIS	76	15.334	0.332	14.487	1.00	19.35	C
ATOM	431	CG	HIS	76	15.229	-0.064	15.924	1.00	18.87	C
ATOM	432	CD2	HIS	76	14.203	0.011	16.804	1.00	20.15	C
ATOM	433	ND1	HIS	76	16.292	-0.602	16.618	1.00	17.09	N
ATOM	434	CE1	HIS	76	15.924	-0.841	17.864	1.00	18.81	C
ATOM	435	NE2	HIS	76	14.661	-0.478	18.003	1.00	18.94	N
ATOM	436	C	HIS	76	16.102	2.027	12.810	1.00	21.94	C
ATOM	437	O	HIS	76	15.285	1.781	11.923	1.00	21.85	O
ATOM	438	N	CYS	77	17.314	2.496	12.567	1.00	19.97	N
ATOM	439	CA	CYS	77	17.743	2.787	11.217	1.00	22.10	C
ATOM	440	CB	CYS	77	17.956	4.292	11.050	1.00	21.37	C
ATOM	441	SG	CYS	77	19.086	4.999	12.249	1.00	23.82	S
ATOM	442	C	CYS	77	18.889	1.937	10.685	1.00	22.14	C
ATOM	443	O	CYS	77	19.434	2.212	9.619	1.00	21.97	O
ATOM	444	N	SER	78	19.251	0.899	11.434	1.00	22.19	N
ATOM	445	CA	SER	78	20.304	-0.010	11.000	1.00	24.97	C
ATOM	446	CB	SER	78	20.689	-1.008	12.098	1.00	24.27	C
ATOM	447	OG	SER	78	19.588	-1.819	12.475	1.00	23.96	O
ATOM	448	C	SER	78	19.746	-0.727	9.772	1.00	27.08	C
ATOM	449	O	SER	78	18.578	-1.098	9.752	1.00	29.90	O
ATOM	450	N	ASN	79	20.562	-0.896	8.739	1.00	31.08	N
ATOM	451	CA	ASN	79	20.102	-1.579	7.530	1.00	33.84	C
ATOM	452	CB	ASN	79	19.475	-2.939	7.856	1.00	37.91	C
ATOM	453	CG	ASN	79	20.430	-3.867	8.583	1.00	40.98	C
ATOM	454	OD1	ASN	79	20.087	-4.436	9.623	1.00	44.02	O
ATOM	455	ND2	ASN	79	21.639	-4.014	8.050	1.00	41.58	N
ATOM	456	C	ASN	79	19.130	-0.741	6.688	1.00	33.03	C
ATOM	457	O	ASN	79	18.500	-1.260	5.765	1.00	31.20	O
ATOM	458	N	ASP	80	18.995	0.543	7.013	1.00	28.81	N
ATOM	459	CA	ASP	80	18.098	1.425	6.263	1.00	24.65	C
ATOM	460	CB	ASP	80	16.875	1.821	7.091	1.00	23.49	C
ATOM	461	CG	ASP	80	15.880	2.662	6.307	1.00	24.25	C
ATOM	462	OD1	ASP	80	14.807	2.115	5.975	1.00	23.20	O
ATOM	463	OD2	ASP	80	16.143	3.855	6.033	1.00	22.88	O
ATOM	464	C	ASP	80	18.890	2.645	5.811	1.00	24.06	C
ATOM	465	O	ASP	80	19.814	3.078	6.505	1.00	21.20	O
ATOM	466	N	LEU	81	18.546	3.188	4.647	1.00	21.17	N
ATOM	467	CA	LEU	81	19.257	4.354	4.131	1.00	23.85	C
ATOM	468	CB	LEU	81	18.638	4.843	2.819	1.00	27.45	C

ATOM	469	CG	LEU	81	19.280	6.057	2.138	1.00	29.98	C
ATOM	470	CD1	LEU	81	20.759	5.822	1.861	1.00	32.81	C
ATOM	471	CD2	LEU	81	18.548	6.421	0.845	1.00	32.11	C
ATOM	472	C	LEU	81	19.313	5.486	5.155	1.00	21.14	C
ATOM	473	O	LEU	81	20.249	6.285	5.156	1.00	18.90	O
ATOM	474	N	LEU	82	18.325	5.546	6.042	1.00	18.54	N
ATOM	475	CA	LEU	82	18.318	6.598	7.052	1.00	15.44	C
ATOM	476	CB	LEU	82	17.019	6.590	7.857	1.00	16.76	C
ATOM	477	CG	LEU	82	16.897	7.650	8.953	1.00	16.50	C
ATOM	478	CD1	LEU	82	17.090	9.052	8.382	1.00	17.63	C
ATOM	479	CD2	LEU	82	15.559	7.538	9.694	1.00	17.39	C
ATOM	480	C	LEU	82	19.516	6.405	7.969	1.00	15.53	C
ATOM	481	O	LEU	82	20.070	7.373	8.495	1.00	15.91	O
ATOM	482	N	GLY	83	19.916	5.145	8.139	1.00	14.77	N
ATOM	483	CA	GLY	83	21.058	4.824	8.978	1.00	16.75	C
ATOM	484	C	GLY	83	22.341	5.353	8.365	1.00	19.93	C
ATOM	485	O	GLY	83	23.236	5.824	9.075	1.00	19.30	O
ATOM	486	N	ASP	84	22.445	5.278	7.042	1.00	20.23	N
ATOM	487	CA	ASP	84	23.640	5.790	6.376	1.00	19.77	C
ATOM	488	CB	ASP	84	23.694	5.372	4.903	1.00	22.40	C
ATOM	489	CG	ASP	84	23.784	3.865	4.712	1.00	24.25	C
ATOM	490	OD1	ASP	84	24.006	3.137	5.700	1.00	24.82	O
ATOM	491	OD2	ASP	84	23.627	3.407	3.562	1.00	25.98	O
ATOM	492	C	ASP	84	23.670	7.314	6.487	1.00	20.03	C
ATOM	493	O	ASP	84	24.739	7.919	6.577	1.00	19.62	O
ATOM	494	N	LEU	85	22.488	7.925	6.511	1.00	16.11	N
ATOM	495	CA	LEU	85	22.385	9.377	6.578	1.00	17.87	C
ATOM	496	CB	LEU	85	20.972	9.827	6.194	1.00	19.94	C
ATOM	497	CG	LEU	85	20.673	11.320	6.099	1.00	24.54	C
ATOM	498	CD1	LEU	85	21.547	11.960	5.019	1.00	24.28	C
ATOM	499	CD2	LEU	85	19.205	11.575	5.770	1.00	24.55	C
ATOM	500	C	LEU	85	22.779	9.897	7.965	1.00	18.33	C
ATOM	501	O	LEU	85	23.522	10.875	8.078	1.00	18.30	O
ATOM	502	N	PHE	86	22.297	9.247	9.022	1.00	18.48	N
ATOM	503	CA	PHE	86	22.646	9.686	10.372	1.00	19.70	C
ATOM	504	CB	PHE	86	21.491	9.506	11.370	1.00	22.89	C
ATOM	505	CG	PHE	86	20.283	10.375	11.092	1.00	25.20	C
ATOM	506	CD1	PHE	86	19.282	10.504	12.051	1.00	29.11	C
ATOM	507	CD2	PHE	86	20.175	11.114	9.915	1.00	26.71	C
ATOM	508	CE1	PHE	86	18.195	11.359	11.850	1.00	26.77	C
ATOM	509	CE2	PHE	86	19.091	11.972	9.702	1.00	28.12	C
ATOM	510	CZ	PHE	86	18.100	12.095	10.673	1.00	28.90	C
ATOM	511	C	PHE	86	23.943	9.104	10.927	1.00	19.63	C
ATOM	512	O	PHE	86	24.464	9.594	11.927	1.00	21.85	O
ATOM	513	N	GLY	87	24.468	8.074	10.272	1.00	18.21	N
ATOM	514	CA	GLY	87	25.713	7.467	10.715	1.00	20.13	C
ATOM	515	C	GLY	87	25.620	6.699	12.021	1.00	22.27	C
ATOM	516	O	GLY	87	26.612	6.552	12.737	1.00	21.79	O
ATOM	517	N	VAL	88	24.425	6.210	12.332	1.00	21.59	N
ATOM	518	CA	VAL	88	24.186	5.448	13.555	1.00	22.13	C
ATOM	519	CB	VAL	88	23.716	6.351	14.724	1.00	22.68	C
ATOM	520	CG1	VAL	88	24.830	7.306	15.130	1.00	24.59	C
ATOM	521	CG2	VAL	88	22.463	7.117	14.324	1.00	23.26	C
ATOM	522	C	VAL	88	23.105	4.408	13.305	1.00	21.88	C
ATOM	523	O	VAL	88	22.271	4.576	12.417	1.00	21.47	O
ATOM	524	N	PRO	89	23.116	3.307	14.074	1.00	22.24	N
ATOM	525	CD	PRO	89	23.982	2.960	15.221	1.00	23.24	C
ATOM	526	CA	PRO	89	22.110	2.267	13.901	1.00	20.32	C
ATOM	527	CB	PRO	89	22.807	1.064	14.503	1.00	23.05	C

ATOM	528	CG	PRO	89	23.286	1.669	15.787	1.00	22.08	C
ATOM	529	C	PRO	89	20.770	2.609	14.559	1.00	20.59	C
ATOM	530	O	PRO	89	19.775	1.912	14.343	1.00	21.33	O
ATOM	531	N	SER	90	20.743	3.688	15.341	1.00	18.62	N
ATOM	532	CA	SER	90	19.520	4.097	16.035	1.00	17.25	C
ATOM	533	CB	SER	90	19.109	3.069	17.085	1.00	18.96	C
ATOM	534	OG	SER	90	20.118	2.972	18.080	1.00	16.15	O
ATOM	535	C	SER	90	19.640	5.447	16.724	1.00	17.40	C
ATOM	536	O	SER	90	20.742	5.937	16.990	1.00	17.90	O
ATOM	537	N	PHE	91	18.486	6.040	17.013	1.00	15.60	N
ATOM	538	CA	PHE	91	18.422	7.308	17.718	1.00	16.44	C
ATOM	539	CB	PHE	91	18.750	8.512	16.828	1.00	19.56	C
ATOM	540	CG	PHE	91	17.793	8.708	15.682	1.00	19.09	C
ATOM	541	CD1	PHE	91	17.923	7.972	14.512	1.00	19.65	C
ATOM	542	CD2	PHE	91	16.756	9.634	15.783	1.00	21.34	C
ATOM	543	CE1	PHE	91	17.033	8.153	13.450	1.00	20.79	C
ATOM	544	CE2	PHE	91	15.858	9.824	14.728	1.00	20.20	C
ATOM	545	CZ	PHE	91	15.999	9.083	13.561	1.00	20.92	C
ATOM	546	C	PHE	91	17.043	7.491	18.334	1.00	18.30	C
ATOM	547	O	PHE	91	16.058	6.939	17.846	1.00	17.76	O
ATOM	548	N	SER	92	16.992	8.248	19.422	1.00	18.06	N
ATOM	549	CA	SER	92	15.737	8.575	20.083	1.00	20.21	C
ATOM	550	CB	SER	92	15.913	8.741	21.587	1.00	20.33	C
ATOM	551	OG	SER	92	14.676	9.099	22.181	1.00	22.41	O
ATOM	552	C	SER	92	15.224	9.875	19.468	1.00	20.17	C
ATOM	553	O	SER	92	16.010	10.786	19.203	1.00	19.59	O
ATOM	554	N	VAL	93	13.920	9.970	19.226	1.00	20.06	N
ATOM	555	CA	VAL	93	13.381	11.198	18.647	1.00	22.08	C
ATOM	556	CB	VAL	93	11.867	11.074	18.371	1.00	21.37	C
ATOM	557	CG1	VAL	93	11.608	9.904	17.429	1.00	22.86	C
ATOM	558	CG2	VAL	93	11.114	10.891	19.681	1.00	22.21	C
ATOM	559	C	VAL	93	13.614	12.384	19.583	1.00	22.18	C
ATOM	560	O	VAL	93	13.425	13.538	19.197	1.00	20.43	O
ATOM	561	N	LYS	94	14.038	12.098	20.811	1.00	24.14	N
ATOM	562	CA	LYS	94	14.298	13.153	21.784	1.00	25.77	C
ATOM	563	CB	LYS	94	14.142	12.620	23.213	1.00	30.87	C
ATOM	564	CG	LYS	94	15.094	11.484	23.557	1.00	36.14	C
ATOM	565	CD	LYS	94	14.888	10.965	24.976	1.00	40.51	C
ATOM	566	CE	LYS	94	13.467	10.431	25.164	1.00	42.75	C
ATOM	567	NZ	LYS	94	13.224	9.907	26.539	1.00	45.31	N
ATOM	568	C	LYS	94	15.677	13.781	21.558	1.00	24.88	C
ATOM	569	O	LYS	94	15.992	14.825	22.129	1.00	24.06	O
ATOM	570	N	GLU	95	16.488	13.144	20.715	1.00	23.25	N
ATOM	571	CA	GLU	95	17.823	13.653	20.393	1.00	22.14	C
ATOM	572	CB	GLU	95	18.761	12.526	19.940	1.00	24.17	C
ATOM	573	CG	GLU	95	19.031	11.470	21.011	1.00	28.59	C
ATOM	574	CD	GLU	95	19.963	10.399	20.463	1.00	31.12	C
ATOM	575	OE1	GLU	95	21.188	10.511	20.674	1.00	33.36	O
ATOM	576	OE2	GLU	95	19.473	9.430	19.847	1.00	32.84	O
ATOM	577	C	GLU	95	17.733	14.757	19.346	1.00	20.96	C
ATOM	578	O	GLU	95	18.275	14.639	18.245	1.00	18.40	O
ATOM	579	N	HIS	96	17.049	15.839	19.702	1.00	20.19	N
ATOM	580	CA	HIS	96	16.860	16.952	18.782	1.00	20.60	C
ATOM	581	CB	HIS	96	16.067	18.089	19.431	1.00	19.83	C
ATOM	582	CG	HIS	96	14.680	17.702	19.844	1.00	21.58	C
ATOM	583	CD2	HIS	96	13.987	16.552	19.675	1.00	22.20	C
ATOM	584	ND1	HIS	96	13.827	18.572	20.490	1.00	24.09	N
ATOM	585	CE1	HIS	96	12.667	17.975	20.700	1.00	23.77	C
ATOM	586	NE2	HIS	96	12.737	16.748	20.215	1.00	24.59	N

ATOM	587	C	HIS	96	18.105	17.502	18.110	1.00	19.96	C
ATOM	588	O	HIS	96	18.112	17.693	16.897	1.00	18.54	O
ATOM	589	N	ARG	97	19.163	17.750	18.873	1.00	19.16	N
ATOM	590	CA	ARG	97	20.343	18.309	18.241	1.00	21.59	C
ATOM	591	CB	ARG	97	21.402	18.733	19.254	1.00	23.22	C
ATOM	592	CG	ARG	97	22.605	19.332	18.560	1.00	26.59	C
ATOM	593	CD	ARG	97	23.697	19.791	19.503	1.00	29.57	C
ATOM	594	NE	ARG	97	24.804	20.353	18.733	1.00	30.13	N
ATOM	595	CZ	ARG	97	25.877	20.930	19.263	1.00	31.77	C
ATOM	596	NH1	ARG	97	25.998	21.028	20.581	1.00	30.89	N
ATOM	597	NH2	ARG	97	26.825	21.417	18.470	1.00	30.56	N
ATOM	598	C	ARG	97	20.927	17.382	17.187	1.00	20.19	C
ATOM	599	O	ARG	97	21.341	17.836	16.124	1.00	18.40	O
ATOM	600	N	LYS	98	20.945	16.083	17.472	1.00	20.40	N
ATOM	601	CA	LYS	98	21.462	15.113	16.512	1.00	22.67	C
ATOM	602	CB	LYS	98	21.499	13.710	17.125	1.00	25.02	C
ATOM	603	CG	LYS	98	21.910	12.590	16.172	1.00	28.25	C
ATOM	604	CD	LYS	98	23.290	12.753	15.572	1.00	32.85	C
ATOM	605	CE	LYS	98	23.591	11.577	14.640	1.00	35.25	C
ATOM	606	NZ	LYS	98	24.936	11.653	13.997	1.00	38.28	N
ATOM	607	C	LYS	98	20.636	15.113	15.232	1.00	21.36	C
ATOM	608	O	LYS	98	21.177	15.067	14.124	1.00	20.55	O
ATOM	609	N	ILE	99	19.322	15.183	15.395	1.00	17.78	N
ATOM	610	CA	ILE	99	18.409	15.168	14.261	1.00	16.72	C
ATOM	611	CB	ILE	99	16.962	15.036	14.757	1.00	18.32	C
ATOM	612	CG2	ILE	99	15.999	15.108	13.577	1.00	18.94	C
ATOM	613	CG1	ILE	99	16.813	13.713	15.522	1.00	18.48	C
ATOM	614	CD1	ILE	99	15.432	13.460	16.100	1.00	20.38	C
ATOM	615	C	ILE	99	18.556	16.406	13.379	1.00	15.71	C
ATOM	616	O	ILE	99	18.745	16.289	12.166	1.00	16.59	O
ATOM	617	N	TYR	100	18.473	17.594	13.970	1.00	16.29	N
ATOM	618	CA	TYR	100	18.649	18.801	13.168	1.00	15.73	C
ATOM	619	CB	TYR	100	18.384	20.081	13.959	1.00	17.85	C
ATOM	620	CG	TYR	100	16.958	20.305	14.383	1.00	18.03	C
ATOM	621	CD1	TYR	100	15.989	20.628	13.433	1.00	18.59	C
ATOM	622	CE1	TYR	100	14.686	20.920	13.805	1.00	19.33	C
ATOM	623	CD2	TYR	100	16.585	20.272	15.724	1.00	19.08	C
ATOM	624	CE2	TYR	100	15.277	20.564	16.108	1.00	20.31	C
ATOM	625	CZ	TYR	100	14.337	20.889	15.142	1.00	20.85	C
ATOM	626	OH	TYR	100	13.047	21.200	15.505	1.00	20.48	O
ATOM	627	C	TYR	100	20.028	18.898	12.547	1.00	17.08	C
ATOM	628	O	TYR	100	20.177	19.362	11.417	1.00	17.10	O
ATOM	629	N	THR	101	21.038	18.455	13.282	1.00	17.01	N
ATOM	630	CA	THR	101	22.395	18.547	12.771	1.00	18.10	C
ATOM	631	CB	THR	101	23.421	18.061	13.818	1.00	19.38	C
ATOM	632	OG1	THR	101	23.409	18.950	14.944	1.00	19.40	O
ATOM	633	CG2	THR	101	24.827	18.023	13.219	1.00	21.23	C
ATOM	634	C	THR	101	22.563	17.729	11.509	1.00	17.82	C
ATOM	635	O	THR	101	23.123	18.207	10.523	1.00	18.96	O
ATOM	636	N	MET	102	22.055	16.503	11.515	1.00	14.87	N
ATOM	637	CA	MET	102	22.241	15.673	10.345	1.00	18.86	C
ATOM	638	CB	MET	102	22.051	14.200	10.687	1.00	22.58	C
ATOM	639	CG	MET	102	22.968	13.769	11.831	1.00	25.73	C
ATOM	640	SD	MET	102	24.729	14.046	11.491	1.00	29.72	S
ATOM	641	CE	MET	102	25.017	12.908	10.134	1.00	26.51	C
ATOM	642	C	MET	102	21.366	16.115	9.181	1.00	17.36	C
ATOM	643	O	MET	102	21.716	15.918	8.019	1.00	17.66	O
ATOM	644	N	ILE	103	20.242	16.746	9.488	1.00	17.10	N
ATOM	645	CA	ILE	103	19.360	17.217	8.432	1.00	18.31	C

ATOM	646	CB	ILE	103	17.971	17.598	9.004	1.00	18.43	C
ATOM	647	CG2	ILE	103	17.137	18.308	7.942	1.00	19.89	C
ATOM	648	CG1	ILE	103	17.261	16.336	9.503	1.00	21.17	C
ATOM	649	CD1	ILE	103	15.895	16.591	10.129	1.00	21.70	C
ATOM	650	C	ILE	103	19.997	18.442	7.778	1.00	18.58	C
ATOM	651	O	ILE	103	20.091	18.521	6.552	1.00	18.12	O
ATOM	652	N	TYR	104	20.461	19.384	8.598	1.00	17.58	N
ATOM	653	CA	TYR	104	21.063	20.600	8.065	1.00	19.92	C
ATOM	654	CB	TYR	104	21.408	21.597	9.171	1.00	17.24	C
ATOM	655	CG	TYR	104	20.245	22.073	10.015	1.00	19.35	C
ATOM	656	CD1	TYR	104	18.920	21.924	9.590	1.00	17.10	C
ATOM	657	CE1	TYR	104	17.861	22.409	10.365	1.00	19.27	C
ATOM	658	CD2	TYR	104	20.479	22.714	11.227	1.00	17.95	C
ATOM	659	CE2	TYR	104	19.439	23.196	12.001	1.00	19.03	C
ATOM	660	CZ	TYR	104	18.137	23.044	11.571	1.00	18.59	C
ATOM	661	OH	TYR	104	17.125	23.531	12.363	1.00	18.75	O
ATOM	662	C	TYR	104	22.308	20.332	7.224	1.00	18.77	C
ATOM	663	O	TYR	104	22.544	21.020	6.234	1.00	17.01	O
ATOM	664	N	ARG	105	23.095	19.332	7.608	1.00	19.90	N
ATOM	665	CA	ARG	105	24.303	19.019	6.850	1.00	22.21	C
ATOM	666	CB	ARG	105	25.252	18.112	7.634	1.00	23.00	C
ATOM	667	CG	ARG	105	25.786	18.721	8.916	1.00	25.04	C
ATOM	668	CD	ARG	105	26.724	17.753	9.616	1.00	27.11	C
ATOM	669	NE	ARG	105	27.269	18.306	10.853	1.00	27.31	N
ATOM	670	CZ	ARG	105	27.986	17.604	11.724	1.00	28.44	C
ATOM	671	NH1	ARG	105	28.241	16.324	11.488	1.00	27.73	N
ATOM	672	NH2	ARG	105	28.442	18.175	12.831	1.00	28.80	N
ATOM	673	C	ARG	105	24.007	18.433	5.479	1.00	22.30	C
ATOM	674	O	ARG	105	24.893	18.333	4.632	1.00	24.22	O
ATOM	675	N	ASN	106	22.752	18.054	5.267	1.00	20.53	N
ATOM	676	CA	ASN	106	22.317	17.497	3.993	1.00	21.19	C
ATOM	677	CB	ASN	106	21.721	16.097	4.143	1.00	19.77	C
ATOM	678	CG	ASN	106	22.744	15.084	4.612	1.00	22.79	C
ATOM	679	OD1	ASN	106	23.423	14.470	3.788	1.00	23.07	O
ATOM	680	ND2	ASN	106	22.843	14.879	5.924	1.00	19.66	N
ATOM	681	C	ASN	106	21.414	18.414	3.191	1.00	20.46	C
ATOM	682	O	ASN	106	20.657	17.967	2.324	1.00	19.35	O
ATOM	683	N	LEU	107	21.510	19.703	3.497	1.00	20.37	N
ATOM	684	CA	LEU	107	20.722	20.740	2.845	1.00	22.38	C
ATOM	685	CB	LEU	107	19.663	21.303	3.791	1.00	21.77	C
ATOM	686	CG	LEU	107	18.618	20.323	4.316	1.00	23.03	C
ATOM	687	CD1	LEU	107	17.706	20.996	5.334	1.00	21.74	C
ATOM	688	CD2	LEU	107	17.816	19.716	3.169	1.00	22.14	C
ATOM	689	C	LEU	107	21.586	21.872	2.306	1.00	23.14	C
ATOM	690	O	LEU	107	22.618	22.205	2.884	1.00	22.39	O
ATOM	691	N	VAL	108	21.158	22.445	1.189	1.00	23.48	N
ATOM	692	CA	VAL	108	21.846	23.569	0.566	1.00	26.51	C
ATOM	693	CB	VAL	108	22.484	23.158	-0.776	1.00	28.25	C
ATOM	694	CG1	VAL	108	23.128	24.364	-1.432	1.00	31.06	C
ATOM	695	CG2	VAL	108	23.511	22.064	-0.543	1.00	30.49	C
ATOM	696	C	VAL	108	20.795	24.647	0.304	1.00	25.74	C
ATOM	697	O	VAL	108	19.766	24.363	-0.301	1.00	24.47	O
ATOM	698	N	VAL	109	21.042	25.871	0.767	1.00	25.08	N
ATOM	699	CA	VAL	109	20.096	26.958	0.548	1.00	28.59	C
ATOM	700	CB	VAL	109	20.491	28.224	1.325	1.00	30.38	C
ATOM	701	CG1	VAL	109	19.457	29.320	1.088	1.00	30.40	C
ATOM	702	CG2	VAL	109	20.600	27.907	2.809	1.00	30.06	C
ATOM	703	C	VAL	109	20.007	27.285	-0.942	1.00	31.09	C
ATOM	704	O	VAL	109	21.017	27.538	-1.600	1.00	30.68	O

ATOM	705	N	VAL	110	18.784	27.273	-1.457	1.00	34.26	N
ATOM	706	CA	VAL	110	18.497	27.512	-2.869	1.00	40.68	C
ATOM	707	CB	VAL	110	17.092	26.951	-3.231	1.00	41.37	C
ATOM	708	CG1	VAL	110	16.776	27.222	-4.691	1.00	42.09	C
ATOM	709	CG2	VAL	110	17.046	25.456	-2.948	1.00	41.30	C
ATOM	710	C	VAL	110	18.586	28.950	-3.376	1.00	45.42	C
ATOM	711	O	VAL	110	18.320	29.904	-2.643	1.00	45.46	O
ATOM	712	N	ASN	111	18.980	29.063	-4.644	1.00	50.62	N
ATOM	713	CA	ASN	111	19.090	30.319	-5.380	1.00	54.41	C
ATOM	714	CB	ASN	111	18.454	30.130	-6.764	1.00	57.01	C
ATOM	715	CG	ASN	111	18.469	31.386	-7.600	1.00	59.53	C
ATOM	716	OD1	ASN	111	17.413	31.886	-7.991	1.00	60.59	O
ATOM	717	ND2	ASN	111	19.660	31.900	-7.891	1.00	60.11	N
ATOM	718	C	ASN	111	18.521	31.547	-4.662	1.00	54.78	C
ATOM	719	O	ASN	111	17.400	31.982	-4.938	1.00	56.01	O
ATOM	720	C1	SCH	996	-1.000	8.674	15.423	1.00	58.94	
ATOM	721	C2	SCH	996	0.074	7.820	16.068	1.00	58.85	
ATOM	722	O1	SCH	996	-0.100	7.261	17.153	1.00	59.47	
ATOM	723	N1	SCH	996	1.202	7.745	15.337	1.00	57.09	
ATOM	724	C3	SCH	996	2.471	7.578	16.042	1.00	55.53	
ATOM	725	C4	SCH	996	3.102	8.922	16.429	1.00	58.30	
ATOM	726	O2	SCH	996	3.020	9.892	15.673	1.00	59.95	
ATOM	727	N2	SCH	996	3.724	8.908	17.631	1.00	61.62	
ATOM	728	C5	SCH	996	4.020	10.154	18.329	1.00	63.29	
ATOM	729	C6	SCH	996	2.706	10.796	18.790	1.00	65.39	
ATOM	730	O3	SCH	996	2.516	12.011	18.716	1.00	65.59	
ATOM	731	N3	SCH	996	1.822	9.901	19.269	1.00	67.92	
ATOM	732	C7	SCH	996	0.554	10.407	19.793	1.00	70.11	
ATOM	733	C8	SCH	996	0.346	9.699	20.930	1.00	70.97	
ATOM	734	O4	SCH	996	0.223	10.153	22.077	1.00	71.51	
ATOM	735	O5	SCH	996	0.325	8.321	20.830	1.00	71.56	
ATOM	736	C9	SCH	996	3.406	6.688	15.192	1.00	49.97	
ATOM	737	C10	SCH	996	-0.504	10.428	18.687	1.00	70.56	
ATOM	738	C11	SCH	996	-0.315	11.537	17.664	1.00	71.51	
ATOM	739	C12	SCH	996	-1.305	12.653	17.941	1.00	72.44	
ATOM	740	O6	SCH	996	-0.991	13.651	18.515	1.00	72.59	
ATOM	741	O7	SCH	996	-2.556	12.416	17.482	1.00	72.61	
ATOM	742	C13	SCH	996	4.852	6.796	15.638	1.00	44.76	
ATOM	743	C14	SCH	996	5.890	7.608	15.051	1.00	41.72	
ATOM	744	C15	SCH	996	7.096	7.390	15.825	1.00	40.91	
ATOM	745	N4	SCH	996	6.776	6.472	16.833	1.00	40.79	
ATOM	746	C16	SCH	996	5.458	6.131	16.715	1.00	43.06	
ATOM	747	C17	SCH	996	5.912	8.510	13.946	1.00	40.02	
ATOM	748	C18	SCH	996	7.115	9.193	13.606	1.00	37.25	
ATOM	749	C19	SCH	996	8.298	8.983	14.370	1.00	36.32	
ATOM	750	C20	SCH	996	8.295	8.082	15.474	1.00	37.47	
ATOM	751	CL1	SCH	996	9.737	9.840	13.965	1.00	29.32	
ATOM	752	C21	SCH	996	4.747	11.137	17.396	1.00	62.87	
ATOM	753	C22	SCH	996	4.877	9.854	19.566	1.00	62.94	
ATOM	754	C23	SCH	996	6.279	9.364	19.188	1.00	62.44	
ATOM	755	C24	SCH	996	6.166	10.673	17.043	1.00	62.33	
ATOM	756	C25	SCH	996	7.006	10.356	18.280	1.00	62.08	
ATOM	757	C1	SCH	998	7.020	14.306	19.529	1.00	32.04	
ATOM	758	C2	SCH	998	8.132	14.220	18.499	1.00	30.99	
ATOM	759	O1	SCH	998	9.294	13.953	18.813	1.00	31.33	
ATOM	760	N1	SCH	998	7.711	14.463	17.243	1.00	28.90	
ATOM	761	C3	SCH	998	8.715	14.626	16.190	1.00	28.20	
ATOM	762	C4	SCH	998	9.050	16.095	15.923	1.00	28.60	
ATOM	763	O2	SCH	998	8.184	16.880	15.527	1.00	28.06	

ATOM	764	N2	SCH	998	10.342	16.402	16.159	1.00	26.21
ATOM	765	C5	SCH	998	10.807	17.775	16.036	1.00	28.33
ATOM	766	C6	SCH	998	9.978	18.673	16.963	1.00	33.18
ATOM	767	O3	SCH	998	9.634	19.808	16.630	1.00	35.64
ATOM	768	N3	SCH	998	9.686	18.088	18.140	1.00	37.61
ATOM	769	C7	SCH	998	8.697	18.731	19.006	1.00	42.54
ATOM	770	C8	SCH	998	9.245	18.545	20.404	1.00	42.96
ATOM	771	O4	SCH	998	9.535	19.446	21.049	1.00	44.48
ATOM	772	O5	SCH	998	9.382	17.262	20.693	1.00	43.12
ATOM	773	C9	SCH	998	8.244	13.892	14.923	1.00	27.77
ATOM	774	C10	SCH	998	7.290	18.240	18.661	1.00	44.92
ATOM	775	C11	SCH	998	6.730	18.812	17.368	1.00	48.30
ATOM	776	C12	SCH	998	5.583	19.752	17.684	1.00	49.87
ATOM	777	O6	SCH	998	5.601	20.902	17.365	1.00	51.11
ATOM	778	O7	SCH	998	4.554	19.175	18.348	1.00	50.51
ATOM	779	C13	SCH	998	9.158	14.149	13.740	1.00	25.37
ATOM	780	C14	SCH	998	10.480	13.618	13.499	1.00	25.02
ATOM	781	C15	SCH	998	10.902	14.099	12.199	1.00	25.73
ATOM	782	N4	SCH	998	9.868	14.895	11.699	1.00	25.53
ATOM	783	C16	SCH	998	8.852	14.918	12.609	1.00	25.55
ATOM	784	C17	SCH	998	11.350	12.777	14.254	1.00	25.02
ATOM	785	C18	SCH	998	12.617	12.399	13.724	1.00	25.30
ATOM	786	C19	SCH	998	13.020	12.858	12.437	1.00	26.55
ATOM	787	C20	SCH	998	12.171	13.711	11.677	1.00	24.81
ATOM	788	CL1	SCH	998	14.529	12.355	11.778	1.00	26.71
ATOM	789	C21	SCH	998	10.639	18.264	14.593	1.00	26.42
ATOM	790	C22	SCH	998	12.279	17.852	16.457	1.00	26.82
ATOM	791	C23	SCH	998	13.181	17.061	15.496	1.00	25.79
ATOM	792	C24	SCH	998	11.554	17.510	13.617	1.00	24.84
ATOM	793	C25	SCH	998	13.025	17.532	14.048	1.00	24.21
ATOM	794	C1	SCH	999	14.304	27.533	13.501	1.00	20.77
ATOM	795	C2	SCH	999	14.911	27.965	12.179	1.00	20.52
ATOM	796	O1	SCH	999	14.277	28.618	11.350	1.00	21.90
ATOM	797	N1	SCH	999	16.183	27.557	12.024	1.00	19.32
ATOM	798	C3	SCH	999	16.883	27.892	10.787	1.00	20.88
ATOM	799	C4	SCH	999	16.080	27.517	9.526	1.00	22.08
ATOM	800	O2	SCH	999	15.657	26.372	9.370	1.00	21.93
ATOM	801	N2	SCH	999	15.894	28.551	8.669	1.00	22.51
ATOM	802	C5	SCH	999	15.213	28.377	7.382	1.00	24.88
ATOM	803	C6	SCH	999	13.709	28.188	7.602	1.00	26.16
ATOM	804	O3	SCH	999	12.953	27.935	6.665	1.00	27.24
ATOM	805	N3	SCH	999	13.318	28.331	8.879	1.00	27.90
ATOM	806	C7	SCH	999	11.886	28.201	9.143	1.00	31.35
ATOM	807	C8	SCH	999	11.241	29.583	9.052	1.00	34.43
ATOM	808	O4	SCH	999	10.006	29.558	9.037	1.00	35.86
ATOM	809	O5	SCH	999	11.994	30.531	8.919	1.00	36.30
ATOM	810	C9	SCH	999	18.277	27.245	10.824	1.00	20.43
ATOM	811	C10	SCH	999	11.653	27.295	10.349	1.00	31.35
ATOM	812	C11	SCH	999	12.066	25.844	10.129	1.00	29.83
ATOM	813	C12	SCH	999	11.716	25.026	11.360	1.00	32.05
ATOM	814	O6	SCH	999	12.531	24.381	11.942	1.00	31.48
ATOM	815	O7	SCH	999	10.416	25.092	11.730	1.00	32.95
ATOM	816	C13	SCH	999	19.093	27.591	9.595	1.00	21.99
ATOM	817	C14	SCH	999	19.603	26.675	8.606	1.00	21.11
ATOM	818	C15	SCH	999	20.326	27.456	7.626	1.00	22.79
ATOM	819	N4	SCH	999	20.243	28.793	8.029	1.00	23.21
ATOM	820	C16	SCH	999	19.518	28.863	9.184	1.00	22.90
ATOM	821	C17	SCH	999	19.512	25.260	8.455	1.00	21.66
ATOM	822	C18	SCH	999	20.126	24.621	7.341	1.00	23.05

ATOM	823	C19	SCH	999	20.833	25.388	6.371	1.00	22.06	
ATOM	824	C20	SCH	999	20.937	26.803	6.511	1.00	22.85	
ATOM	825	CL1	SCH	999	21.550	24.594	5.014	1.00	26.59	
ATOM	826	C21	SCH	999	15.747	27.140	6.639	1.00	23.66	
ATOM	827	C22	SCH	999	15.409	29.639	6.533	1.00	24.90	
ATOM	828	C23	SCH	999	16.867	29.820	6.129	1.00	24.75	
ATOM	829	C24	SCH	999	17.176	27.321	6.103	1.00	23.22	
ATOM	830	C25	SCH	999	17.379	28.625	5.329	1.00	23.88	
ATOM	831	OH2	WAT	1001	3.395	9.112	-1.435	1.00	16.59	0
ATOM	839	OH2	WAT	1002	24.775	12.618	6.376	1.00	17.52	0
ATOM	832	OH2	WAT	1003	18.514	-0.561	14.622	1.00	18.25	0
ATOM	835	OH2	WAT	1004	15.044	24.301	11.181	1.00	18.80	0
ATOM	856	OH2	WAT	1005	20.867	15.283	20.436	1.00	24.20	0
ATOM	842	OH2	WAT	1006	9.724	26.049	6.031	1.00	24.72	0
ATOM	860	OH2	WAT	1007	6.074	13.931	10.197	1.00	24.81	0
ATOM	857	OH2	WAT	1008	21.757	8.195	18.608	1.00	25.80	0
ATOM	833	OH2	WAT	1009	12.107	26.746	4.500	1.00	25.85	0
ATOM	843	OH2	WAT	1010	16.620	1.807	2.942	1.00	26.33	0
ATOM	870	OH2	WAT	1011	7.466	20.307	9.569	1.00	26.55	0
ATOM	848	OH2	WAT	1012	11.574	22.642	13.779	1.00	26.80	0
ATOM	834	OH2	WAT	1013	13.759	-1.012	20.605	1.00	28.49	0
ATOM	840	OH2	WAT	1014	25.743	20.101	15.817	1.00	28.57	0
ATOM	855	OH2	WAT	1015	0.544	11.546	5.002	1.00	29.35	0
ATOM	844	OH2	WAT	1016	21.356	8.460	22.469	1.00	30.69	0
ATOM	836	OH2	WAT	1017	14.502	-0.707	6.212	1.00	31.40	0
ATOM	841	OH2	WAT	1018	11.775	14.532	17.517	1.00	31.42	0
ATOM	851	OH2	WAT	1019	22.475	1.722	7.609	1.00	31.99	0
ATOM	859	OH2	WAT	1020	5.732	18.551	-1.162	1.00	32.12	0
ATOM	872	OH2	WAT	1021	23.725	20.929	22.606	1.00	34.79	0
ATOM	862	OH2	WAT	1022	23.860	26.433	1.644	1.00	34.96	0
ATOM	863	OH2	WAT	1023	5.394	16.356	11.571	1.00	35.71	0
ATOM	861	OH2	WAT	1024	21.332	30.796	5.811	1.00	36.70	0
ATOM	847	OH2	WAT	1025	9.148	-2.863	15.900	1.00	37.64	0
ATOM	858	OH2	WAT	1026	14.166	16.525	23.729	1.00	37.84	0
ATOM	866	OH2	WAT	1027	11.334	30.280	5.618	1.00	38.35	0
ATOM	864	OH2	WAT	1028	18.007	18.195	-4.362	1.00	40.37	0
ATOM	871	OH2	WAT	1029	11.860	21.767	17.854	1.00	40.71	0
ATOM	874	OH2	WAT	1030	8.276	27.446	8.045	1.00	41.07	0
ATOM	846	OH2	WAT	1031	7.632	18.152	11.360	1.00	42.63	0
ATOM	865	OH2	WAT	1032	25.898	10.512	16.626	1.00	43.37	0
ATOM	854	OH2	WAT	1033	8.159	-1.042	14.070	1.00	44.00	0
ATOM	852	OH2	WAT	1034	28.464	14.740	9.077	1.00	46.88	0
ATOM	845	OH2	WAT	1035	2.078	6.181	4.658	1.00	47.60	0
ATOM	838	OH2	WAT	1036	26.484	15.271	4.182	1.00	47.99	0
ATOM	867	OH2	WAT	1037	23.657	28.439	-1.144	1.00	50.33	0
ATOM	837	OH2	WAT	1038	13.538	21.361	20.180	1.00	54.13	0
ATOM	869	OH2	WAT	1039	25.590	16.309	16.444	1.00	54.19	0
ATOM	850	OH2	WAT	1040	26.845	13.685	16.859	1.00	55.86	0
ATOM	873	OH2	WAT	1041	27.978	4.166	14.080	1.00	55.87	0
ATOM	868	OH2	WAT	1042	20.489	1.898	-2.662	1.00	56.16	0
ATOM	853	OH2	WAT	1043	17.178	10.875	-6.741	1.00	59.20	0
ATOM	849	OH2	WAT	1044	25.481	2.906	8.796	1.00	63.46	0

END

EXAMPLE 3: Preparation and crystallization of Hdm2 Y76H-Tripeptide Complex*Production and crystallization of modified Hdm2(Y76H):*

The modified Hdm2(Y76H) protein was produced using the QuickChange site-directed mutagenesis method as discussed in Example 1 except that only 5 the primers for Y76H were used in mutagenesis. The p53 peptide analog, Ac-^{6Cl}WAC_{3c}E, disclosed and defined above, was dissolved in the same buffer and added to the Hdm2(Y76H) protein solution.

The single mutant HDM2 (17-125) Y76H-tripeptide complex was crystallized using a hanging-drop vapor diffusion method. The protein-peptide 10 solution (1 μ l; 6-10 mg/ml) in buffer A was mixed with an equal volume of precipitant [0.1 M Tris, pH 8-9, 35% PEG 4000, and 0.0 to 0.2 M magnesium chloride], placed on the underside of a siliconized glass coverslip and sealed in close proximity to 1 ml of the precipitant solution. Vapor diffusion crystallization experiments were conducted using the hanging drop method. Specifically,, 15 crystals were grown from a droplet containing a mixture of 0.5-2.0 μ l of protein and 0.5-1.0 μ l of the precipitant solution. Crystallization plates were incubated at 4° C; rectangular rod crystals (0.1 x 0.1 x 0.3 mm) grew over 2-30 days.

Prior to data collection, crystals were washed with the reservoir solution of the crystallization setup and transferred into the same solution with 10% glycerol 20 added. The crystals were then flash-cooled in a nitrogen stream at 95 K. X-ray diffraction was collected using a Riga generator equipped with a Praxis 4++ detector. Data were integrated and scaled using the HKL package.

Data collection statistics:

25	Resolution	50.0 – .20 Å
	No. of collected reflections	13234
	No. of unique reflections ($F \geq 0$)	4341
	R-sym	6.2%
	Percent of theoretical ($I/s \geq 1$)	84.8%
30	Unit Cell	$a=41.1 \text{ \AA}$, $b=42.7 \text{ \AA}$, $c=53.777 \text{ \AA}$, $\alpha=\beta=\gamma=90^\circ$
	Space Group	P2 ₁ 2 ₁ 2 ₁
	Asymmetric unit	1 molecule

The crystal structure was solved using molecular replacement using the search models 1YCQ and 1YCR from the PDB. Refinement was done using the program CNX.

5	Theoretical number of reflections	5854
	Resolution Limits	50.0 – 2.1 Å
	Number of unobserved reflections	1529(26.1%)
	Number of reflections in working set	4325 (73.9%)
	Number of reflections in test set	197 (3.4%)
10	Number of protein residues	87
	Number of solvent atoms	0
	R-factor	0.45
	R-free	0.51
	RMSD bond length	0.014 Å
	RMSD bond angles	1.97°
15	The structural coordinates for the above-described Hdm2 crystal are set forth below in Table 4.	

TABLE 4

1	2	3	4	5	6	7	8	9	10	11
ATOM	1	CB	GLU	25	7.387	-0.822	5.902	1.00	31.40	C
ATOM	2	CG	GLU	25	7.019	-0.298	4.509	1.00	35.01	C
ATOM	3	CD	GLU	25	7.873	-0.972	3.442	1.00	37.01	C
ATOM	4	OE1	GLU	25	7.321	-1.714	2.600	1.00	35.86	O
ATOM	5	OE2	GLU	25	9.101	-0.744	3.437	1.00	38.68	O
ATOM	6	C	GLU	25	5.081	-0.176	6.662	1.00	30.27	C
ATOM	7	O	GLU	25	4.374	-1.161	6.879	1.00	31.21	O
ATOM	8	N	GLU	25	6.922	-0.708	8.375	1.00	29.47	N
ATOM	9	CA	GLU	25	6.567	-0.166	7.021	1.00	31.52	C
ATOM	10	N	THR	26	4.610	0.938	6.116	1.00	28.18	N
ATOM	11	CA	THR	26	3.217	1.047	5.711	1.00	24.93	C
ATOM	12	CB	THR	26	2.441	2.031	6.611	1.00	24.28	C
ATOM	13	OG1	THR	26	2.654	1.706	7.990	1.00	23.56	O
ATOM	14	CG2	THR	26	0.955	1.959	6.304	1.00	22.48	C
ATOM	15	C	THR	26	3.096	1.533	4.264	1.00	24.10	C
ATOM	16	O	THR	26	3.633	2.581	3.908	1.00	22.83	O
ATOM	17	N	LEU	27	2.405	0.760	3.431	1.00	19.92	N
ATOM	18	CA	LEU	27	2.177	1.160	2.047	1.00	20.55	C
ATOM	19	CB	LEU	27	2.052	-0.044	1.107	1.00	22.12	C
ATOM	20	CG	LEU	27	3.257	-0.991	1.005	1.00	24.55	C
ATOM	21	CD1	LEU	27	2.945	-2.199	0.113	1.00	22.27	C
ATOM	22	CD2	LEU	27	4.506	-0.259	0.513	1.00	22.59	C
ATOM	23	C	LEU	27	0.899	1.995	2.057	1.00	19.40	C
ATOM	24	O	LEU	27	-0.124	1.568	2.599	1.00	18.49	O
ATOM	25	N	VAL	28	0.956	3.190	1.476	1.00	20.26	N
ATOM	26	CA	VAL	28	-0.206	4.075	1.457	1.00	21.18	C
ATOM	27	CB	VAL	28	-0.090	5.200	2.523	1.00	19.01	C
ATOM	28	CG1	VAL	28	0.094	4.599	3.897	1.00	19.71	C
ATOM	29	CG2	VAL	28	1.057	6.125	2.183	1.00	20.08	C
ATOM	30	C	VAL	28	-0.481	4.741	0.118	1.00	21.98	C
ATOM	31	O	VAL	28	0.431	4.966	-0.679	1.00	23.19	O
ATOM	32	N	ARG	29	-1.755	5.042	-0.120	1.00	23.38	N
ATOM	33	CA	ARG	29	-2.188	5.722	-1.338	1.00	25.82	C
ATOM	34	CB	ARG	29	-3.239	4.904	-2.089	1.00	27.99	C
ATOM	35	CG	ARG	29	-2.761	3.515	-2.489	1.00	30.54	C
ATOM	36	CD	ARG	29	-3.838	2.738	-3.220	1.00	33.91	C
ATOM	37	NE	ARG	29	-3.398	1.395	-3.590	1.00	37.13	N
ATOM	38	CZ	ARG	29	-2.478	1.134	-4.511	1.00	39.21	C
ATOM	39	NH1	ARG	29	-1.896	2.131	-5.168	1.00	41.39	N
ATOM	40	NH2	ARG	29	-2.135	-0.122	-4.771	1.00	39.25	N
ATOM	41	C	ARG	29	-2.743	7.082	-0.905	1.00	26.04	C
ATOM	42	O	ARG	29	-3.723	7.163	-0.162	1.00	25.87	O
ATOM	43	N	PRO	30	-2.100	8.167	-1.358	1.00	25.80	N
ATOM	44	CD	PRO	30	-0.679	8.055	-1.738	1.00	24.67	C
ATOM	45	CA	PRO	30	-2.394	9.579	-1.111	1.00	22.90	C
ATOM	46	CB	PRO	30	-1.203	10.259	-1.751	1.00	22.35	C
ATOM	47	CG	PRO	30	-0.114	9.428	-1.255	1.00	26.65	C
ATOM	48	C	PRO	30	-3.699	10.094	-1.723	1.00	22.50	C
ATOM	49	O	PRO	30	-4.062	9.701	-2.835	1.00	21.62	O
ATOM	50	N	LYS	31	-4.411	10.954	-1.001	1.00	22.08	N
ATOM	51	CA	LYS	31	-5.593	11.574	-1.590	1.00	23.03	C
ATOM	52	CB	LYS	31	-6.451	12.381	-0.620	1.00	25.31	C
ATOM	53	CG	LYS	31	-7.122	11.655	0.501	1.00	27.67	C
ATOM	54	CD	LYS	31	-7.906	12.701	1.266	1.00	29.82	C
ATOM	55	CE	LYS	31	-8.654	12.122	2.423	1.00	30.22	C

ATOM	56	NZ	LYS	31	-9.401	13.196	3.133	1.00	31.71	N
ATOM	57	C	LYS	31	-5.065	12.527	-2.648	1.00	21.77	C
ATOM	58	O	LYS	31	-3.906	12.951	-2.590	1.00	21.83	O
ATOM	59	N	PRO	32	-5.909	12.873	-3.629	1.00	20.24	N
ATOM	60	CD	PRO	32	-7.253	12.301	-3.838	1.00	20.22	C
ATOM	61	CA	PRO	32	-5.608	13.770	-4.736	1.00	19.66	C
ATOM	62	CB	PRO	32	-6.996	14.120	-5.227	1.00	21.15	C
ATOM	63	CG	PRO	32	-7.598	12.787	-5.277	1.00	21.31	C
ATOM	64	C	PRO	32	-4.799	15.018	-4.348	1.00	18.96	C
ATOM	65	O	PRO	32	-3.832	15.368	-5.022	1.00	17.76	O
ATOM	66	N	LEU	33	-5.190	15.682	-3.263	1.00	20.65	N
ATOM	67	CA	LEU	33	-4.497	16.901	-2.853	1.00	22.91	C
ATOM	68	CB	LEU	33	-5.412	17.805	-2.025	1.00	27.03	C
ATOM	69	CG	LEU	33	-6.690	18.264	-2.733	1.00	30.44	C
ATOM	70	CD1	LEU	33	-7.567	19.092	-1.796	1.00	32.45	C
ATOM	71	CD2	LEU	33	-6.385	19.036	-4.014	1.00	31.10	C
ATOM	72	C	LEU	33	-3.172	16.659	-2.141	1.00	22.13	C
ATOM	73	O	LEU	33	-2.277	17.507	-2.169	1.00	20.13	O
ATOM	74	N	LEU	34	-3.041	15.500	-1.507	1.00	22.44	N
ATOM	75	CA	LEU	34	-1.788	15.170	-0.845	1.00	22.32	C
ATOM	76	CB	LEU	34	-1.967	14.032	0.158	1.00	22.46	C
ATOM	77	CG	LEU	34	-0.686	13.562	0.851	1.00	25.03	C
ATOM	78	CD1	LEU	34	0.092	14.709	1.488	1.00	22.68	C
ATOM	79	CD2	LEU	34	-0.984	12.449	1.846	1.00	25.15	C
ATOM	80	C	LEU	34	-0.833	14.775	-1.959	1.00	20.98	C
ATOM	81	O	LEU	34	0.370	15.027	-1.892	1.00	18.72	O
ATOM	82	N	LEU	35	-1.403	14.172	-2.996	1.00	22.16	N
ATOM	83	CA	LEU	35	-0.644	13.746	-4.158	1.00	22.50	C
ATOM	84	CB	LEU	35	-1.499	12.888	-5.086	1.00	21.79	C
ATOM	85	CG	LEU	35	-0.799	12.451	-6.371	1.00	21.31	C
ATOM	86	CD1	LEU	35	0.509	11.738	-6.069	1.00	22.29	C
ATOM	87	CD2	LEU	35	-1.709	11.605	-7.249	1.00	21.81	C
ATOM	88	C	LEU	35	-0.120	14.950	-4.913	1.00	23.62	C
ATOM	89	O	LEU	35	0.965	14.909	-5.489	1.00	24.50	O
ATOM	90	N	LYS	36	-0.892	16.029	-4.887	1.00	26.45	N
ATOM	91	CA	LYS	36	-0.517	17.247	-5.581	1.00	30.02	C
ATOM	92	CB	LYS	36	-1.739	18.156	-5.759	1.00	31.51	C
ATOM	93	CG	LYS	36	-1.468	19.454	-6.494	1.00	34.42	C
ATOM	94	CD	LYS	36	-2.742	20.280	-6.633	1.00	37.59	C
ATOM	95	CE	LYS	36	-2.483	21.584	-7.381	1.00	40.02	C
ATOM	96	NZ	LYS	36	-3.723	22.409	-7.540	1.00	42.11	N
ATOM	97	C	LYS	36	0.624	17.964	-4.864	1.00	30.99	C
ATOM	98	O	LYS	36	1.321	18.782	-5.460	1.00	32.96	O
ATOM	99	N	LEU	37	0.828	17.645	-3.590	1.00	31.87	N
ATOM	100	CA	LEU	37	1.909	18.274	-2.841	1.00	33.15	C
ATOM	101	CB	LEU	37	1.504	18.569	-1.395	1.00	34.78	C
ATOM	102	CG	LEU	37	0.316	19.498	-1.144	1.00	37.22	C
ATOM	103	CD1	LEU	37	0.039	19.625	0.347	1.00	36.79	C
ATOM	104	CD2	LEU	37	0.540	20.873	-1.760	1.00	38.28	C
ATOM	105	C	LEU	37	3.153	17.395	-2.866	1.00	32.75	C
ATOM	106	O	LEU	37	4.277	17.889	-2.747	1.00	31.66	O
ATOM	107	N	LEU	38	2.945	16.093	-3.040	1.00	30.45	N
ATOM	108	CA	LEU	38	4.054	15.155	-3.080	1.00	28.31	C
ATOM	109	CB	LEU	38	3.578	13.726	-2.805	1.00	27.70	C
ATOM	110	CG	LEU	38	2.878	13.360	-1.492	1.00	27.23	C
ATOM	111	CD1	LEU	38	2.383	11.925	-1.547	1.00	27.09	C
ATOM	112	CD2	LEU	38	3.750	13.597	-0.262	1.00	24.95	C
ATOM	113	C	LEU	38	4.713	15.230	-4.451	1.00	28.49	C
ATOM	114	O	LEU	38	5.888	14.897	-4.598	1.00	29.16	O

ATOM	115	N	LYS	39	3.956	15.684	-5.449	1.00	27.07	N
ATOM	116	CA	LYS	39	4.480	15.783	-6.806	1.00	28.73	C
ATOM	117	CB	LYS	39	3.373	15.754	-7.861	1.00	33.27	C
ATOM	118	CG	LYS	39	2.504	14.521	-7.920	1.00	37.75	C
ATOM	119	CD	LYS	39	1.487	14.719	-9.035	1.00	41.51	C
ATOM	120	CE	LYS	39	0.550	13.539	-9.187	1.00	45.31	C
ATOM	121	NZ	LYS	39	-0.430	13.767	-10.292	1.00	47.13	N
ATOM	122	C	LYS	39	5.304	17.036	-7.034	1.00	25.77	C
ATOM	123	O	LYS	39	6.154	17.062	-7.916	1.00	27.98	O
ATOM	124	N	SER	40	5.064	18.071	-6.240	1.00	24.34	N
ATOM	125	CA	SER	40	5.795	19.311	-6.427	1.00	23.75	C
ATOM	126	CB	SER	40	5.033	20.501	-5.845	1.00	24.25	C
ATOM	127	OG	SER	40	4.844	20.354	-4.451	1.00	24.47	O
ATOM	128	C	SER	40	7.180	19.229	-5.825	1.00	23.75	C
ATOM	129	O	SER	40	8.029	20.066	-6.099	1.00	26.76	O
ATOM	130	N	VAL	41	7.414	18.203	-5.018	1.00	26.35	N
ATOM	131	CA	VAL	41	8.701	18.055	-4.367	1.00	25.90	C
ATOM	132	CB	VAL	41	8.550	18.299	-2.846	1.00	26.80	C
ATOM	133	CG1	VAL	41	7.800	17.139	-2.204	1.00	25.77	C
ATOM	134	CG2	VAL	41	9.905	18.491	-2.202	1.00	28.50	C
ATOM	135	C	VAL	41	9.308	16.674	-4.597	1.00	26.12	C
ATOM	136	O	VAL	41	10.480	16.450	-4.300	1.00	28.42	O
ATOM	137	N	GLY	42	8.523	15.751	-5.144	1.00	24.94	N
ATOM	138	CA	GLY	42	9.049	14.416	-5.363	1.00	22.59	C
ATOM	139	C	GLY	42	8.438	13.615	-6.495	1.00	22.27	C
ATOM	140	O	GLY	42	7.720	14.143	-7.347	1.00	19.19	O
ATOM	141	N	ALA	43	8.740	12.321	-6.490	1.00	21.23	N
ATOM	142	CA	ALA	43	8.252	11.387	-7.491	1.00	23.12	C
ATOM	143	CB	ALA	43	8.939	10.043	-7.326	1.00	22.78	C
ATOM	144	C	ALA	43	6.740	11.202	-7.448	1.00	26.26	C
ATOM	145	O	ALA	43	6.137	11.093	-6.382	1.00	26.47	O
ATOM	146	N	GLN	44	6.144	11.182	-8.632	1.00	29.51	N
ATOM	147	CA	GLN	44	4.711	10.994	-8.814	1.00	34.61	C
ATOM	148	CB	GLN	44	4.254	11.798	-10.044	1.00	38.60	C
ATOM	149	CG	GLN	44	2.772	11.752	-10.418	1.00	44.52	C
ATOM	150	CD	GLN	44	2.343	10.341	-10.743	1.00	47.57	C
ATOM	151	OE1	GLN	44	1.459	9.781	-10.093	1.00	50.77	O
ATOM	152	NE2	GLN	44	2.959	9.760	-11.768	1.00	49.38	N
ATOM	153	C	GLN	44	4.452	9.486	-8.949	1.00	33.65	C
ATOM	154	O	GLN	44	4.970	8.844	-9.857	1.00	34.09	O
ATOM	155	N	LYS	45	3.660	8.925	-8.040	1.00	33.88	N
ATOM	156	CA	LYS	45	3.353	7.493	-8.071	1.00	34.47	C
ATOM	157	CB	LYS	45	4.586	6.652	-7.744	1.00	31.48	C
ATOM	158	CG	LYS	45	5.205	6.911	-6.388	1.00	28.37	C
ATOM	159	CD	LYS	45	6.411	6.017	-6.214	1.00	24.70	C
ATOM	160	CE	LYS	45	7.084	6.228	-4.883	1.00	24.73	C
ATOM	161	NZ	LYS	45	8.267	5.329	-4.749	1.00	24.48	N
ATOM	162	C	LYS	45	2.151	7.096	-7.218	1.00	36.41	C
ATOM	163	O	LYS	45	1.924	7.670	-6.157	1.00	36.79	O
ATOM	164	N	ASP	46	1.375	6.120	-7.693	1.00	39.70	N
ATOM	165	CA	ASP	46	0.178	5.678	-6.974	1.00	41.06	C
ATOM	166	CB	ASP	46	-0.641	4.672	-7.792	1.00	42.39	C
ATOM	167	CG	ASP	46	-1.137	5.241	-9.105	1.00	43.83	C
ATOM	168	OD1	ASP	46	-0.864	4.624	-10.156	1.00	45.18	O
ATOM	169	OD2	ASP	46	-1.799	6.301	-9.086	1.00	43.47	O
ATOM	170	C	ASP	46	0.391	5.118	-5.573	1.00	40.30	C
ATOM	171	O	ASP	46	-0.539	5.103	-4.769	1.00	40.91	O
ATOM	172	N	THR	47	1.605	4.671	-5.268	1.00	38.48	N
ATOM	173	CA	THR	47	1.868	4.100	-3.953	1.00	35.27	C

ATOM	174	CB	THR	47	1.860	2.564	-4.000	1.00	34.96	C
ATOM	175	OG1	THR	47	0.592	2.115	-4.482	1.00	35.07	O
ATOM	176	CG2	THR	47	2.083	1.986	-2.614	1.00	34.48	C
ATOM	177	C	THR	47	3.165	4.561	-3.312	1.00	33.33	C
ATOM	178	O	THR	47	4.224	4.569	-3.942	1.00	34.15	O
ATOM	179	N	TYR	48	3.055	4.951	-2.048	1.00	30.30	N
ATOM	180	CA	TYR	48	4.180	5.435	-1.256	1.00	27.10	C
ATOM	181	CB	TYR	48	4.048	6.925	-0.949	1.00	24.18	C
ATOM	182	CG	TYR	48	3.995	7.837	-2.150	1.00	21.88	C
ATOM	183	CD1	TYR	48	5.152	8.418	-2.659	1.00	19.95	C
ATOM	184	CE1	TYR	48	5.096	9.298	-3.727	1.00	20.60	C
ATOM	185	CD2	TYR	48	2.782	8.153	-2.747	1.00	19.91	C
ATOM	186	CE2	TYR	48	2.716	9.027	-3.812	1.00	20.90	C
ATOM	187	CZ	TYR	48	3.872	9.598	-4.298	1.00	20.62	C
ATOM	188	OH	TYR	48	3.795	10.481	-5.347	1.00	22.83	O
ATOM	189	C	TYR	48	4.269	4.685	0.061	1.00	26.72	C
ATOM	190	O	TYR	48	3.275	4.158	0.558	1.00	27.60	O
ATOM	191	N	THR	49	5.470	4.620	0.616	1.00	27.57	N
ATOM	192	CA	THR	49	5.647	4.001	1.916	1.00	27.42	C
ATOM	193	CB	THR	49	7.100	3.558	2.171	1.00	26.03	C
ATOM	194	OG1	THR	49	7.953	4.708	2.191	1.00	24.83	O
ATOM	195	CG2	THR	49	7.562	2.595	1.092	1.00	26.14	C
ATOM	196	C	THR	49	5.317	5.167	2.836	1.00	26.91	C
ATOM	197	O	THR	49	5.606	6.313	2.506	1.00	27.92	O
ATOM	198	N	MET	50	4.692	4.891	3.969	1.00	28.56	N
ATOM	199	CA	MET	50	4.345	5.953	4.905	1.00	30.04	C
ATOM	200	CB	MET	50	3.773	5.358	6.190	1.00	28.54	C
ATOM	201	CG	MET	50	3.355	6.367	7.246	1.00	30.69	C
ATOM	202	SD	MET	50	2.058	7.489	6.688	1.00	30.76	S
ATOM	203	CE	MET	50	2.888	9.022	6.804	1.00	31.08	C
ATOM	204	C	MET	50	5.605	6.746	5.233	1.00	30.79	C
ATOM	205	O	MET	50	5.546	7.896	5.664	1.00	31.44	O
ATOM	206	N	LYS	51	6.753	6.132	4.992	1.00	31.31	N
ATOM	207	CA	LYS	51	8.008	6.764	5.343	1.00	31.72	C
ATOM	208	CB	LYS	51	8.996	5.654	5.711	1.00	34.50	C
ATOM	209	CG	LYS	51	10.325	6.042	6.283	1.00	39.52	C
ATOM	210	CD	LYS	51	11.086	4.747	6.577	1.00	43.25	C
ATOM	211	CE	LYS	51	12.449	4.991	7.201	1.00	46.68	C
ATOM	212	NZ	LYS	51	12.318	5.719	8.503	1.00	47.10	N
ATOM	213	C	LYS	51	8.503	7.745	4.265	1.00	28.34	C
ATOM	214	O	LYS	51	9.297	8.637	4.559	1.00	25.46	O
ATOM	215	N	GLU	52	8.011	7.600	3.032	1.00	24.36	N
ATOM	216	CA	GLU	52	8.388	8.523	1.956	1.00	23.10	C
ATOM	217	CB	GLU	52	8.166	7.936	0.560	1.00	23.72	C
ATOM	218	CG	GLU	52	8.895	6.653	0.213	1.00	26.58	C
ATOM	219	CD	GLU	52	8.521	6.256	-1.209	1.00	25.01	C
ATOM	220	OE1	GLU	52	7.629	5.400	-1.371	1.00	24.55	O
ATOM	221	OE2	GLU	52	9.123	6.785	-2.164	1.00	25.12	O
ATOM	222	C	GLU	52	7.468	9.738	2.088	1.00	21.67	C
ATOM	223	O	GLU	52	7.843	10.860	1.740	1.00	18.74	O
ATOM	224	N	VAL	53	6.263	9.495	2.599	1.00	18.59	N
ATOM	225	CA	VAL	53	5.268	10.545	2.763	1.00	19.55	C
ATOM	226	CB	VAL	53	3.896	9.949	3.143	1.00	19.33	C
ATOM	227	CG1	VAL	53	2.896	11.062	3.434	1.00	19.57	C
ATOM	228	CG2	VAL	53	3.394	9.067	2.016	1.00	20.00	C
ATOM	229	C	VAL	53	5.703	11.518	3.841	1.00	19.68	C
ATOM	230	O	VAL	53	5.559	12.727	3.684	1.00	19.09	O
ATOM	231	N	LEU	54	6.253	10.983	4.926	1.00	21.56	N
ATOM	232	CA	LEU	54	6.732	11.811	6.022	1.00	24.05	C

ATOM	233	CB	LEU	54	7.118	10.949	7.227	1.00	25.58	C
ATOM	234	CG	LEU	54	5.962	10.130	7.805	1.00	27.53	C
ATOM	235	CD1	LEU	54	6.406	9.184	8.917	1.00	27.26	C
ATOM	236	CD2	LEU	54	4.854	11.055	8.278	1.00	27.23	C
ATOM	237	C	LEU	54	7.917	12.628	5.530	1.00	23.85	C
ATOM	238	O	LEU	54	8.066	13.800	5.876	1.00	24.62	O
ATOM	239	N	PHE	55	8.747	12.002	4.702	1.00	22.58	N
ATOM	240	CA	PHE	55	9.908	12.674	4.145	1.00	21.31	C
ATOM	241	CB	PHE	55	10.796	11.716	3.357	1.00	20.68	C
ATOM	242	CG	PHE	55	11.981	12.387	2.725	1.00	20.30	C
ATOM	243	CD1	PHE	55	13.012	12.888	3.507	1.00	18.41	C
ATOM	244	CD2	PHE	55	12.048	12.549	1.346	1.00	19.84	C
ATOM	245	CE1	PHE	55	14.085	13.538	2.928	1.00	18.68	C
ATOM	246	CE2	PHE	55	13.120	13.201	0.759	1.00	18.51	C
ATOM	247	CZ	PHE	55	14.140	13.696	1.552	1.00	19.56	C
ATOM	248	C	PHE	55	9.512	13.850	3.269	1.00	20.62	C
ATOM	249	O	PHE	55	9.963	14.971	3.493	1.00	23.48	O
ATOM	250	N	TYR	56	8.670	13.599	2.270	1.00	19.21	N
ATOM	251	CA	TYR	56	8.243	14.671	1.382	1.00	17.94	C
ATOM	252	CB	TYR	56	7.459	14.142	0.182	1.00	17.19	C
ATOM	253	CG	TYR	56	8.259	13.265	-0.752	1.00	17.01	C
ATOM	254	CD1	TYR	56	9.447	13.726	-1.310	1.00	16.35	C
ATOM	255	CE1	TYR	56	10.130	12.987	-2.251	1.00	15.96	C
ATOM	256	CD2	TYR	56	7.781	12.024	-1.158	1.00	17.74	C
ATOM	257	CE2	TYR	56	8.457	11.273	-2.105	1.00	18.06	C
ATOM	258	CZ	TYR	56	9.627	11.763	-2.651	1.00	18.15	C
ATOM	259	OH	TYR	56	10.268	11.055	-3.637	1.00	17.16	O
ATOM	260	C	TYR	56	7.506	15.830	2.034	1.00	18.40	C
ATOM	261	O	TYR	56	7.708	16.974	1.632	1.00	20.22	O
ATOM	262	N	LEU	57	6.666	15.559	3.034	1.00	18.61	N
ATOM	263	CA	LEU	57	5.964	16.652	3.699	1.00	19.85	C
ATOM	264	CB	LEU	57	4.869	16.162	4.655	1.00	20.61	C
ATOM	265	CG	LEU	57	3.644	15.424	4.115	1.00	23.15	C
ATOM	266	CD1	LEU	57	2.757	14.911	5.243	1.00	23.67	C
ATOM	267	CD2	LEU	57	2.843	16.320	3.162	1.00	21.07	C
ATOM	268	C	LEU	57	6.942	17.560	4.425	1.00	19.93	C
ATOM	269	O	LEU	57	6.778	18.777	4.429	1.00	20.07	O
ATOM	270	N	GLY	58	7.970	16.968	5.023	1.00	18.96	N
ATOM	271	CA	GLY	58	8.959	17.764	5.724	1.00	19.61	C
ATOM	272	C	GLY	58	9.735	18.612	4.736	1.00	20.66	C
ATOM	273	O	GLY	58	9.973	19.801	4.968	1.00	19.89	O
ATOM	274	N	GLN	59	10.132	18.003	3.622	1.00	21.02	N
ATOM	275	CA	GLN	59	10.873	18.730	2.600	1.00	22.78	C
ATOM	276	CB	GLN	59	11.519	17.776	1.599	1.00	22.59	C
ATOM	277	CG	GLN	59	12.505	16.839	2.250	1.00	25.03	C
ATOM	278	CD	GLN	59	13.576	17.674	2.912	1.00	26.69	C
ATOM	279	OE1	GLN	59	13.877	17.502	4.090	1.00	28.42	O
ATOM	280	NE2	GLN	59	14.139	18.612	2.160	1.00	27.94	N
ATOM	281	C	GLN	59	9.980	19.745	1.900	1.00	21.11	C
ATOM	282	O	GLN	59	10.458	20.730	1.342	1.00	20.60	O
ATOM	283	N	TYR	60	8.675	19.504	1.943	1.00	20.96	N
ATOM	284	CA	TYR	60	7.725	20.427	1.340	1.00	21.51	C
ATOM	285	CB	TYR	60	6.350	19.786	1.207	1.00	20.18	C
ATOM	286	CG	TYR	60	5.290	20.727	0.684	1.00	19.26	C
ATOM	287	CD1	TYR	60	5.127	20.946	-0.677	1.00	18.65	C
ATOM	288	CE1	TYR	60	4.145	21.796	-1.148	1.00	18.43	C
ATOM	289	CD2	TYR	60	4.444	21.390	1.563	1.00	18.15	C
ATOM	290	CE2	TYR	60	3.465	22.238	1.106	1.00	19.04	C
ATOM	291	CZ	TYR	60	3.314	22.437	-0.251	1.00	19.66	C

ATOM	292	OH	TYR	60	2.316	23.266	-0.705	1.00	17.66	O
ATOM	293	C	TYR	60	7.631	21.661	2.221	1.00	21.23	C
ATOM	294	O	TYR	60	7.592	22.793	1.737	1.00	21.02	O
ATOM	295	N	ILE	61	7.615	21.414	3.525	1.00	20.45	N
ATOM	296	CA	ILE	61	7.514	22.463	4.518	1.00	19.88	C
ATOM	297	CB	ILE	61	7.305	21.841	5.913	1.00	16.63	C
ATOM	298	CG2	ILE	61	7.405	22.907	6.990	1.00	15.17	C
ATOM	299	CG1	ILE	61	5.961	21.112	5.956	1.00	14.67	C
ATOM	300	CD1	ILE	61	5.660	20.431	7.295	1.00	13.50	C
ATOM	301	C	ILE	61	8.789	23.293	4.518	1.00	24.11	C
ATOM	302	O	ILE	61	8.779	24.463	4.915	1.00	24.81	O
ATOM	303	N	MET	62	9.882	22.695	4.052	1.00	27.53	N
ATOM	304	CA	MET	62	11.158	23.399	4.010	1.00	31.34	C
ATOM	305	CB	MET	62	12.331	22.454	4.293	1.00	34.73	C
ATOM	306	CG	MET	62	12.295	21.791	5.665	1.00	41.21	C
ATOM	307	SD	MET	62	13.705	20.685	5.949	1.00	45.94	S
ATOM	308	CE	MET	62	15.034	21.883	6.155	1.00	46.15	C
ATOM	309	C	MET	62	11.394	24.154	2.710	1.00	30.78	C
ATOM	310	O	MET	62	11.975	25.236	2.723	1.00	31.93	O
ATOM	311	N	THR	63	10.929	23.607	1.591	1.00	30.29	N
ATOM	312	CA	THR	63	11.146	24.278	0.318	1.00	31.93	C
ATOM	313	CB	THR	63	10.913	23.331	-0.879	1.00	32.45	C
ATOM	314	OG1	THR	63	11.653	22.121	-0.691	1.00	34.57	O
ATOM	315	CG2	THR	63	11.383	23.992	-2.168	1.00	33.58	C
ATOM	316	C	THR	63	10.222	25.476	0.164	1.00	30.66	C
ATOM	317	O	THR	63	10.550	26.430	-0.535	1.00	32.12	O
ATOM	318	N	LYS	64	9.072	25.437	0.825	1.00	30.47	N
ATOM	319	CA	LYS	64	8.144	26.554	0.750	1.00	30.88	C
ATOM	320	CB	LYS	64	6.715	26.113	0.433	1.00	31.95	C
ATOM	321	CG	LYS	64	6.592	25.412	-0.903	1.00	33.47	C
ATOM	322	CD	LYS	64	5.163	25.021	-1.188	1.00	35.44	C
ATOM	323	CE	LYS	64	4.269	26.251	-1.223	1.00	35.32	C
ATOM	324	NZ	LYS	64	2.851	25.905	-1.507	1.00	36.49	N
ATOM	325	C	LYS	64	8.218	27.370	2.021	1.00	30.38	C
ATOM	326	O	LYS	64	7.504	28.356	2.190	1.00	31.61	O
ATOM	327	N	ARG	65	9.096	26.929	2.914	1.00	31.08	N
ATOM	328	CA	ARG	65	9.350	27.604	4.176	1.00	32.41	C
ATOM	329	CB	ARG	65	10.296	28.776	3.921	1.00	33.62	C
ATOM	330	CG	ARG	65	11.569	28.220	3.304	1.00	38.45	C
ATOM	331	CD	ARG	65	12.660	29.194	2.949	1.00	39.91	C
ATOM	332	NE	ARG	65	13.766	28.425	2.375	1.00	42.85	N
ATOM	333	CZ	ARG	65	14.793	28.944	1.713	1.00	43.34	C
ATOM	334	NH1	ARG	65	14.878	30.255	1.530	1.00	45.53	N
ATOM	335	NH2	ARG	65	15.729	28.146	1.221	1.00	43.64	N
ATOM	336	C	ARG	65	8.099	27.982	4.955	1.00	30.80	C
ATOM	337	O	ARG	65	7.856	29.145	5.259	1.00	28.75	O
ATOM	338	N	LEU	66	7.310	26.965	5.274	1.00	31.27	N
ATOM	339	CA	LEU	66	6.077	27.149	6.020	1.00	31.53	C
ATOM	340	CB	LEU	66	5.082	26.044	5.687	1.00	30.56	C
ATOM	341	CG	LEU	66	4.742	25.973	4.201	1.00	30.97	C
ATOM	342	CD1	LEU	66	3.800	24.809	3.914	1.00	31.85	C
ATOM	343	CD2	LEU	66	4.156	27.290	3.700	1.00	28.41	C
ATOM	344	C	LEU	66	6.391	27.171	7.507	1.00	32.00	C
ATOM	345	O	LEU	66	5.505	27.320	8.342	1.00	36.99	O
ATOM	346	N	TYR	67	7.669	27.018	7.822	1.00	32.16	N
ATOM	347	CA	TYR	67	8.147	27.010	9.196	1.00	29.34	C
ATOM	348	CB	TYR	67	9.300	26.023	9.365	1.00	30.19	C
ATOM	349	CG	TYR	67	10.505	26.362	8.517	1.00	30.32	C
ATOM	350	CD1	TYR	67	11.362	27.393	8.877	1.00	31.21	C

ATOM	351	CE1	TYR	67	12.453	27.726	8.097	1.00	31.23	C
ATOM	352	CD2	TYR	67	10.775	25.668	7.348	1.00	31.49	C
ATOM	353	CE2	TYR	67	11.867	25.995	6.559	1.00	31.76	C
ATOM	354	CZ	TYR	67	12.702	27.025	6.939	1.00	30.71	C
ATOM	355	OH	TYR	67	13.790	27.351	6.163	1.00	30.69	O
ATOM	356	C	TYR	67	8.584	28.410	9.599	1.00	26.76	C
ATOM	357	O	TYR	67	8.974	29.212	8.755	1.00	27.18	O
ATOM	358	N	ASP	68	8.505	28.710	10.887	1.00	26.00	N
ATOM	359	CA	ASP	68	8.931	30.012	11.376	1.00	24.98	C
ATOM	360	CB	ASP	68	8.536	30.213	12.832	1.00	24.44	C
ATOM	361	CG	ASP	68	8.916	31.581	13.354	1.00	25.82	C
ATOM	362	OD1	ASP	68	8.229	32.064	14.280	1.00	27.13	O
ATOM	363	OD2	ASP	68	9.876	32.189	12.829	1.00	23.05	O
ATOM	364	C	ASP	68	10.456	30.065	11.239	1.00	26.08	C
ATOM	365	O	ASP	68	11.171	29.230	11.809	1.00	20.81	O
ATOM	366	N	GLU	69	10.954	31.038	10.481	1.00	26.25	N
ATOM	367	CA	GLU	69	12.393	31.179	10.299	1.00	27.50	C
ATOM	368	CB	GLU	69	12.711	32.341	9.355	1.00	31.03	C
ATOM	369	CG	GLU	69	14.200	32.606	9.160	1.00	36.70	C
ATOM	370	CD	GLU	69	14.885	31.383	8.579	1.00	40.21	C
ATOM	371	OE1	GLU	69	14.189	30.407	8.224	1.00	42.79	O
ATOM	372	OE2	GLU	69	16.133	31.400	8.486	1.00	41.52	O
ATOM	373	C	GLU	69	13.155	31.330	11.617	1.00	25.94	C
ATOM	374	O	GLU	69	14.320	30.956	11.705	1.00	25.86	O
ATOM	375	N	LYS	70	12.493	31.856	12.645	1.00	23.46	N
ATOM	376	CA	LYS	70	13.141	32.048	13.937	1.00	23.45	C
ATOM	377	CB	LYS	70	12.882	33.449	14.490	1.00	26.70	C
ATOM	378	CG	LYS	70	13.362	34.567	13.582	1.00	28.84	C
ATOM	379	CD	LYS	70	14.858	34.477	13.329	1.00	31.67	C
ATOM	380	CE	LYS	70	15.327	35.607	12.415	1.00	33.30	C
ATOM	381	NZ	LYS	70	16.788	35.549	12.150	1.00	34.03	N
ATOM	382	C	LYS	70	12.762	30.989	14.965	1.00	22.06	C
ATOM	383	O	LYS	70	13.147	31.077	16.123	1.00	21.58	O
ATOM	384	N	GLN	71	11.995	29.993	14.541	1.00	21.15	N
ATOM	385	CA	GLN	71	11.588	28.913	15.436	1.00	20.20	C
ATOM	386	CB	GLN	71	10.497	29.383	16.407	1.00	20.24	C
ATOM	387	CG	GLN	71	10.110	28.357	17.460	1.00	20.50	C
ATOM	388	CD	GLN	71	9.029	28.928	18.363	1.00	20.46	C
ATOM	389	OE1	GLN	71	8.365	29.908	18.019	1.00	24.60	O
ATOM	390	NE2	GLN	71	8.865	28.330	19.535	1.00	18.17	N
ATOM	391	C	GLN	71	11.088	27.853	14.461	1.00	20.18	C
ATOM	392	O	GLN	71	9.880	27.643	14.280	1.00	20.12	O
ATOM	393	N	GLN	72	12.056	27.188	13.840	1.00	20.81	N
ATOM	394	CA	GLN	72	11.806	26.189	12.813	1.00	22.77	C
ATOM	395	CB	GLN	72	13.119	25.855	12.110	1.00	23.53	C
ATOM	396	CG	GLN	72	13.723	27.086	11.456	1.00	27.22	C
ATOM	397	CD	GLN	72	15.027	26.744	10.776	1.00	29.38	C
ATOM	398	OE1	GLN	72	15.497	25.604	10.824	1.00	30.31	O
ATOM	399	NE2	GLN	72	15.626	27.741	10.137	1.00	30.16	N
ATOM	400	C	GLN	72	11.028	24.940	13.195	1.00	21.80	C
ATOM	401	O	GLN	72	10.603	24.178	12.328	1.00	20.25	O
ATOM	402	N	HIS	73	10.829	24.737	14.488	1.00	20.18	N
ATOM	403	CA	HIS	73	10.076	23.585	14.949	1.00	21.21	C
ATOM	404	CB	HIS	73	10.627	23.080	16.287	1.00	22.75	C
ATOM	405	CG	HIS	73	10.662	24.117	17.366	1.00	24.88	C
ATOM	406	CD2	HIS	73	11.670	24.911	17.801	1.00	25.16	C
ATOM	407	ND1	HIS	73	9.565	24.435	18.136	1.00	26.05	N
ATOM	408	CE1	HIS	73	9.895	25.378	19.001	1.00	27.37	C
ATOM	409	NE2	HIS	73	11.167	25.684	18.818	1.00	27.33	N

ATOM	410	C	HIS	73	8.569	23.857	14.955	1.00	19.30	C
ATOM	411	O	HIS	73	7.762	23.018	15.353	1.00	15.05	O
ATOM	412	N	ILE	74	8.202	25.049	14.495	1.00	20.68	N
ATOM	413	CA	ILE	74	6.800	25.442	14.408	1.00	20.38	C
ATOM	414	CB	ILE	74	6.517	26.761	15.170	1.00	17.27	C
ATOM	415	CG2	ILE	74	5.082	27.186	14.930	1.00	16.94	C
ATOM	416	CG1	ILE	74	6.788	26.585	16.673	1.00	12.85	C
ATOM	417	CD1	ILE	74	5.940	25.522	17.350	1.00	10.25	C
ATOM	418	C	ILE	74	6.433	25.631	12.931	1.00	22.21	C
ATOM	419	O	ILE	74	7.068	26.401	12.207	1.00	19.54	O
ATOM	420	N	VAL	75	5.406	24.914	12.489	1.00	22.66	N
ATOM	421	CA	VAL	75	4.968	24.994	11.106	1.00	20.85	C
ATOM	422	CB	VAL	75	4.656	23.596	10.552	1.00	19.84	C
ATOM	423	CG1	VAL	75	4.268	23.697	9.081	1.00	17.74	C
ATOM	424	CG2	VAL	75	5.870	22.678	10.745	1.00	19.97	C
ATOM	425	C	VAL	75	3.730	25.866	10.931	1.00	22.04	C
ATOM	426	O	VAL	75	2.682	25.605	11.523	1.00	19.88	O
ATOM	427	N	HIS	76	3.860	26.907	10.117	1.00	19.99	N
ATOM	428	CA	HIS	76	2.742	27.797	9.850	1.00	20.86	C
ATOM	429	CB	HIS	76	3.140	29.249	10.074	1.00	20.76	C
ATOM	430	CG	HIS	76	3.595	29.532	11.471	1.00	18.48	C
ATOM	431	CD2	HIS	76	4.799	29.914	11.958	1.00	17.24	C
ATOM	432	ND1	HIS	76	2.764	29.401	12.561	1.00	18.78	N
ATOM	433	CE1	HIS	76	3.436	29.693	13.661	1.00	17.42	C
ATOM	434	NE2	HIS	76	4.673	30.007	13.323	1.00	17.51	N
ATOM	435	C	HIS	76	2.250	27.541	8.429	1.00	22.01	C
ATOM	436	O	HIS	76	2.906	27.886	7.448	1.00	20.06	O
ATOM	437	N	CYS	77	1.076	26.934	8.333	1.00	25.10	N
ATOM	438	CA	CYS	77	0.503	26.582	7.049	1.00	27.53	C
ATOM	439	CB	CYS	77	0.499	25.055	6.882	1.00	26.53	C
ATOM	440	SG	CYS	77	-0.321	24.144	8.202	1.00	29.08	S
ATOM	441	C	CYS	77	-0.805	27.255	6.632	1.00	28.98	C
ATOM	442	O	CYS	77	-1.442	26.851	5.660	1.00	28.62	O
ATOM	443	N	SER	78	-1.206	28.283	7.377	1.00	31.46	N
ATOM	444	CA	SER	78	-2.404	29.043	7.035	1.00	34.18	C
ATOM	445	CB	SER	78	-2.859	29.936	8.186	1.00	32.67	C
ATOM	446	OG	SER	78	-1.858	30.878	8.515	1.00	32.54	O
ATOM	447	C	SER	78	-1.935	29.883	5.851	1.00	37.37	C
ATOM	448	O	SER	78	-0.941	30.602	5.966	1.00	40.89	O
ATOM	449	N	ASN	79	-2.637	29.782	4.725	1.00	38.16	N
ATOM	450	CA	ASN	79	-2.281	30.491	3.490	1.00	38.25	C
ATOM	451	CB	ASN	79	-1.490	31.788	3.729	1.00	41.43	C
ATOM	452	CG	ASN	79	-2.269	32.814	4.535	1.00	44.32	C
ATOM	453	OD1	ASN	79	-1.804	33.293	5.568	1.00	47.01	O
ATOM	454	ND2	ASN	79	-3.464	33.155	4.062	1.00	45.45	N
ATOM	455	C	ASN	79	-1.535	29.579	2.522	1.00	36.47	C
ATOM	456	O	ASN	79	-1.050	30.026	1.481	1.00	36.50	O
ATOM	457	N	ASP	80	-1.439	28.301	2.879	1.00	33.49	N
ATOM	458	CA	ASP	80	-0.782	27.304	2.038	1.00	30.65	C
ATOM	459	CB	ASP	80	0.597	26.908	2.567	1.00	28.08	C
ATOM	460	CG	ASP	80	1.300	25.894	1.666	1.00	27.51	C
ATOM	461	OD1	ASP	80	2.347	26.248	1.088	1.00	28.18	O
ATOM	462	OD2	ASP	80	0.818	24.749	1.528	1.00	24.49	O
ATOM	463	C	ASP	80	-1.696	26.089	1.926	1.00	30.18	C
ATOM	464	O	ASP	80	-2.399	25.750	2.876	1.00	32.29	O
ATOM	465	N	LEU	81	-1.701	25.447	0.763	1.00	29.44	N
ATOM	466	CA	LEU	81	-2.547	24.279	0.548	1.00	28.49	C
ATOM	467	CB	LEU	81	-2.242	23.632	-0.806	1.00	30.75	C
ATOM	468	CG	LEU	81	-3.069	22.396	-1.179	1.00	32.89	C

ATOM	469	CD1	LEU	81	-4.564	22.720	-1.167	1.00	32.68	C
ATOM	470	CD2	LEU	81	-2.658	21.821	-2.530	1.00	33.32	C
ATOM	471	C	LEU	81	-2.428	23.240	1.667	1.00	27.48	C
ATOM	472	O	LEU	81	-3.327	22.422	1.861	1.00	26.60	O
ATOM	473	N	LEU	82	-1.328	23.284	2.411	1.00	25.83	N
ATOM	474	CA	LEU	82	-1.121	22.326	3.485	1.00	25.93	C
ATOM	475	CB	LEU	82	0.335	22.334	3.953	1.00	27.37	C
ATOM	476	CG	LEU	82	0.714	21.341	5.051	1.00	26.40	C
ATOM	477	CD1	LEU	82	0.391	19.918	4.617	1.00	28.04	C
ATOM	478	CD2	LEU	82	2.183	21.459	5.440	1.00	25.48	C
ATOM	479	C	LEU	82	-2.075	22.622	4.640	1.00	26.61	C
ATOM	480	O	LEU	82	-2.394	21.736	5.434	1.00	24.04	O
ATOM	481	N	GLY	83	-2.539	23.870	4.713	1.00	25.97	N
ATOM	482	CA	GLY	83	-3.476	24.255	5.751	1.00	23.73	C
ATOM	483	C	GLY	83	-4.816	23.613	5.437	1.00	24.97	C
ATOM	484	O	GLY	83	-5.531	23.137	6.324	1.00	23.63	O
ATOM	485	N	ASP	84	-5.155	23.592	4.154	1.00	26.10	N
ATOM	486	CA	ASP	84	-6.404	22.994	3.705	1.00	28.17	C
ATOM	487	CB	ASP	84	-6.695	23.378	2.259	1.00	29.18	C
ATOM	488	CG	ASP	84	-6.829	24.868	2.065	1.00	31.27	C
ATOM	489	OD1	ASP	84	-6.896	25.604	3.073	1.00	30.35	O
ATOM	490	OD2	ASP	84	-6.856	25.298	0.891	1.00	33.57	O
ATOM	491	C	ASP	84	-6.345	21.477	3.817	1.00	28.68	C
ATOM	492	O	ASP	84	-7.369	20.818	3.992	1.00	29.33	O
ATOM	493	N	LEU	85	-5.138	20.931	3.728	1.00	28.47	N
ATOM	494	CA	LEU	85	-4.946	19.491	3.787	1.00	28.25	C
ATOM	495	CB	LEU	85	-3.572	19.113	3.231	1.00	29.46	C
ATOM	496	CG	LEU	85	-3.224	17.628	3.151	1.00	31.51	C
ATOM	497	CD1	LEU	85	-4.226	16.913	2.258	1.00	31.47	C
ATOM	498	CD2	LEU	85	-1.809	17.393	2.634	1.00	32.07	C
ATOM	499	C	LEU	85	-5.125	19.006	5.229	1.00	27.80	C
ATOM	500	O	LEU	85	-5.923	18.105	5.500	1.00	26.59	O
ATOM	501	N	PHE	86	-4.386	19.618	6.149	1.00	25.85	N
ATOM	502	CA	PHE	86	-4.466	19.262	7.561	1.00	25.42	C
ATOM	503	CB	PHE	86	-3.153	19.569	8.288	1.00	25.61	C
ATOM	504	CG	PHE	86	-1.984	18.745	7.833	1.00	24.56	C
ATOM	505	CD1	PHE	86	-0.723	18.972	8.362	1.00	25.69	C
ATOM	506	CD2	PHE	86	-2.158	17.687	6.955	1.00	25.22	C
ATOM	507	CE1	PHE	86	0.343	18.158	8.033	1.00	24.12	C
ATOM	508	CE2	PHE	86	-1.096	16.866	6.620	1.00	24.92	C
ATOM	509	CZ	PHE	86	0.156	17.100	7.161	1.00	25.70	C
ATOM	510	C	PHE	86	-5.626	19.871	8.342	1.00	25.22	C
ATOM	511	O	PHE	86	-5.984	19.374	9.403	1.00	27.68	O
ATOM	512	N	GLY	87	-6.220	20.936	7.821	1.00	25.48	N
ATOM	513	CA	GLY	87	-7.308	21.572	8.538	1.00	24.46	C
ATOM	514	C	GLY	87	-6.846	22.301	9.793	1.00	24.27	C
ATOM	515	O	GLY	87	-7.577	22.374	10.778	1.00	25.23	O
ATOM	516	N	VAL	88	-5.628	22.835	9.763	1.00	23.15	N
ATOM	517	CA	VAL	88	-5.067	23.582	10.892	1.00	21.50	C
ATOM	518	CB	VAL	88	-4.183	22.697	11.837	1.00	22.47	C
ATOM	519	CG1	VAL	88	-5.014	21.573	12.444	1.00	23.92	C
ATOM	520	CG2	VAL	88	-2.985	22.136	11.082	1.00	21.60	C
ATOM	521	C	VAL	88	-4.200	24.704	10.341	1.00	19.98	C
ATOM	522	O	VAL	88	-3.544	24.541	9.317	1.00	19.52	O
ATOM	523	N	PRO	89	-4.196	25.871	11.001	1.00	21.92	N
ATOM	524	CD	PRO	89	-4.796	26.279	12.289	1.00	20.40	C
ATOM	525	CA	PRO	89	-3.366	26.958	10.488	1.00	21.32	C
ATOM	526	CB	PRO	89	-4.009	28.164	11.135	1.00	21.47	C
ATOM	527	CG	PRO	89	-4.109	27.664	12.548	1.00	21.05	C

ATOM	528	C	PRO	89	-1.881	26.800	10.808	1.00	21.71	C
ATOM	529	O	PRO	89	-1.029	27.350	10.114	1.00	20.80	O
ATOM	530	N	SER	90	-1.579	26.025	11.845	1.00	22.01	N
ATOM	531	CA	SER	90	-0.197	25.803	12.257	1.00	24.39	C
ATOM	532	CB	SER	90	0.475	27.107	12.704	1.00	25.95	C
ATOM	533	OG	SER	90	-0.191	27.688	13.806	1.00	30.18	O
ATOM	534	C	SER	90	-0.076	24.728	13.329	1.00	23.46	C
ATOM	535	O	SER	90	-1.048	24.407	14.010	1.00	25.46	O
ATOM	536	N	PHE	91	1.119	24.161	13.462	1.00	23.68	N
ATOM	537	CA	PHE	91	1.375	23.126	14.463	1.00	22.17	C
ATOM	538	CB	PHE	91	0.869	21.755	14.002	1.00	22.00	C
ATOM	539	CG	PHE	91	1.527	21.265	12.739	1.00	23.50	C
ATOM	540	CD1	PHE	91	1.133	21.751	11.497	1.00	23.67	C
ATOM	541	CD2	PHE	91	2.575	20.360	12.798	1.00	21.96	C
ATOM	542	CE1	PHE	91	1.775	21.342	10.339	1.00	22.42	C
ATOM	543	CE2	PHE	91	3.220	19.949	11.645	1.00	23.74	C
ATOM	544	CZ	PHE	91	2.818	20.442	10.414	1.00	22.47	C
ATOM	545	C	PHE	91	2.862	23.032	14.812	1.00	21.03	C
ATOM	546	O	PHE	91	3.700	23.714	14.225	1.00	22.64	O
ATOM	547	N	SER	92	3.162	22.177	15.781	1.00	18.50	N
ATOM	548	CA	SER	92	4.517	21.909	16.234	1.00	17.97	C
ATOM	549	CB	SER	92	4.626	21.948	17.750	1.00	17.24	C
ATOM	550	OG	SER	92	5.941	21.622	18.147	1.00	15.01	O
ATOM	551	C	SER	92	4.938	20.538	15.718	1.00	19.41	C
ATOM	552	O	SER	92	4.134	19.607	15.711	1.00	16.66	O
ATOM	553	N	VAL	93	6.186	20.417	15.273	1.00	19.68	N
ATOM	554	CA	VAL	93	6.676	19.142	14.766	1.00	20.81	C
ATOM	555	CB	VAL	93	8.052	19.290	14.079	1.00	22.21	C
ATOM	556	CG1	VAL	93	8.008	20.418	13.061	1.00	21.22	C
ATOM	557	CG2	VAL	93	9.134	19.522	15.119	1.00	20.18	C
ATOM	558	C	VAL	93	6.819	18.131	15.897	1.00	21.81	C
ATOM	559	O	VAL	93	7.136	16.969	15.662	1.00	24.55	O
ATOM	560	N	LYS	94	6.579	18.568	17.127	1.00	22.80	N
ATOM	561	CA	LYS	94	6.696	17.670	18.267	1.00	23.16	C
ATOM	562	CB	LYS	94	7.400	18.364	19.431	1.00	25.99	C
ATOM	563	CG	LYS	94	6.681	19.571	19.966	1.00	29.97	C
ATOM	564	CD	LYS	94	7.486	20.206	21.088	1.00	34.85	C
ATOM	565	CE	LYS	94	8.855	20.641	20.553	1.00	34.82	C
ATOM	566	NZ	LYS	94	9.710	21.284	21.583	1.00	37.75	N
ATOM	567	C	LYS	94	5.343	17.088	18.669	1.00	20.95	C
ATOM	568	O	LYS	94	5.253	16.252	19.570	1.00	19.08	O
ATOM	569	N	GLU	95	4.297	17.538	17.983	1.00	19.27	N
ATOM	570	CA	GLU	95	2.937	17.055	18.212	1.00	18.96	C
ATOM	571	CB	GLU	95	1.919	18.145	17.873	1.00	18.81	C
ATOM	572	CG	GLU	95	2.046	19.387	18.743	1.00	21.00	C
ATOM	573	CD	GLU	95	1.017	20.418	18.319	1.00	22.03	C
ATOM	574	OE1	GLU	95	-0.095	20.444	18.888	1.00	20.78	O
ATOM	575	OE2	GLU	95	1.338	21.228	17.429	1.00	23.38	O
ATOM	576	C	GLU	95	2.751	15.823	17.334	1.00	18.97	C
ATOM	577	O	GLU	95	2.008	15.853	16.359	1.00	17.30	O
ATOM	578	N	HIS	96	3.426	14.735	17.698	1.00	20.49	N
ATOM	579	CA	HIS	96	3.374	13.503	16.919	1.00	22.53	C
ATOM	580	CB	HIS	96	4.357	12.468	17.460	1.00	22.80	C
ATOM	581	CG	HIS	96	5.778	12.924	17.429	1.00	22.99	C
ATOM	582	CD2	HIS	96	6.331	14.078	16.987	1.00	24.26	C
ATOM	583	ND1	HIS	96	6.818	12.157	17.903	1.00	22.37	N
ATOM	584	CE1	HIS	96	7.952	12.818	17.754	1.00	25.34	C
ATOM	585	NE2	HIS	96	7.683	13.987	17.200	1.00	25.87	N
ATOM	586	C	HIS	96	2.016	12.865	16.703	1.00	23.09	C

ATOM	587	O	HIS	96	1.741	12.375	15.611	1.00	25.80	O
ATOM	588	N	ARG	97	1.153	12.871	17.712	1.00	23.57	N
ATOM	589	CA	ARG	97	-0.145	12.253	17.509	1.00	22.11	C
ATOM	590	CB	ARG	97	-0.852	11.940	18.829	1.00	22.67	C
ATOM	591	CG	ARG	97	-2.215	11.295	18.608	1.00	25.29	C
ATOM	592	CD	ARG	97	-2.946	10.947	19.895	1.00	24.16	C
ATOM	593	NE	ARG	97	-4.238	10.344	19.586	1.00	25.87	N
ATOM	594	CZ	ARG	97	-5.067	9.819	20.481	1.00	25.09	C
ATOM	595	NH1	ARG	97	-4.748	9.801	21.769	1.00	25.97	N
ATOM	596	NH2	ARG	97	-6.236	9.339	20.086	1.00	24.37	N
ATOM	597	C	ARG	97	-1.019	13.095	16.596	1.00	20.82	C
ATOM	598	O	ARG	97	-1.837	12.563	15.849	1.00	22.99	O
ATOM	599	N	LYS	98	-0.833	14.409	16.638	1.00	19.91	N
ATOM	600	CA	LYS	98	-1.614	15.294	15.787	1.00	19.73	C
ATOM	601	CB	LYS	98	-1.487	16.749	16.228	1.00	20.60	C
ATOM	602	CG	LYS	98	-2.252	17.723	15.331	1.00	23.23	C
ATOM	603	CD	LYS	98	-2.091	19.164	15.794	1.00	23.00	C
ATOM	604	CE	LYS	98	-2.611	19.344	17.205	1.00	24.33	C
ATOM	605	NZ	LYS	98	-2.463	20.740	17.674	1.00	25.97	N
ATOM	606	C	LYS	98	-1.181	15.160	14.331	1.00	18.95	C
ATOM	607	O	LYS	98	-2.013	15.142	13.423	1.00	18.06	O
ATOM	608	N	ILE	99	0.126	15.047	14.120	1.00	16.71	N
ATOM	609	CA	ILE	99	0.664	14.935	12.777	1.00	18.14	C
ATOM	610	CB	ILE	99	2.205	15.049	12.794	1.00	18.88	C
ATOM	611	CG2	ILE	99	2.764	14.887	11.382	1.00	17.52	C
ATOM	612	CG1	ILE	99	2.612	16.408	13.372	1.00	16.52	C
ATOM	613	CD1	ILE	99	4.102	16.610	13.445	1.00	18.05	C
ATOM	614	C	ILE	99	0.252	13.632	12.105	1.00	19.52	C
ATOM	615	O	ILE	99	-0.096	13.633	10.929	1.00	20.84	O
ATOM	616	N	TYR	100	0.278	12.520	12.836	1.00	21.34	N
ATOM	617	CA	TYR	100	-0.140	11.258	12.234	1.00	21.76	C
ATOM	618	CB	TYR	100	0.388	10.029	12.982	1.00	20.52	C
ATOM	619	CG	TYR	100	1.880	9.828	12.897	1.00	21.94	C
ATOM	620	CD1	TYR	100	2.456	9.359	11.724	1.00	22.57	C
ATOM	621	CE1	TYR	100	3.812	9.131	11.633	1.00	22.83	C
ATOM	622	CD2	TYR	100	2.708	10.070	13.982	1.00	22.53	C
ATOM	623	CE2	TYR	100	4.068	9.847	13.901	1.00	22.55	C
ATOM	624	CZ	TYR	100	4.613	9.374	12.724	1.00	22.83	C
ATOM	625	OH	TYR	100	5.962	9.125	12.642	1.00	24.71	O
ATOM	626	C	TYR	100	-1.637	11.164	12.051	1.00	22.16	C
ATOM	627	O	TYR	100	-2.120	10.375	11.240	1.00	20.79	O
ATOM	628	N	THR	101	-2.375	11.980	12.789	1.00	22.43	N
ATOM	629	CA	THR	101	-3.818	11.949	12.659	1.00	20.93	C
ATOM	630	CB	THR	101	-4.501	12.622	13.867	1.00	20.70	C
ATOM	631	OG1	THR	101	-4.220	11.862	15.049	1.00	21.16	O
ATOM	632	CG2	THR	101	-6.013	12.703	13.661	1.00	19.19	C
ATOM	633	C	THR	101	-4.176	12.684	11.382	1.00	20.33	C
ATOM	634	O	THR	101	-4.980	12.199	10.583	1.00	19.32	O
ATOM	635	N	MET	102	-3.551	13.843	11.186	1.00	20.81	N
ATOM	636	CA	MET	102	-3.803	14.664	10.006	1.00	21.77	C
ATOM	637	CB	MET	102	-3.128	16.031	10.119	1.00	22.96	C
ATOM	638	CG	MET	102	-3.563	16.875	11.305	1.00	25.26	C
ATOM	639	SD	MET	102	-2.691	18.457	11.303	1.00	26.02	S
ATOM	640	CE	MET	102	-1.054	17.961	11.843	1.00	25.10	C
ATOM	641	C	MET	102	-3.326	13.982	8.735	1.00	21.84	C
ATOM	642	O	MET	102	-3.959	14.101	7.689	1.00	22.57	O
ATOM	643	N	ILE	103	-2.215	13.259	8.828	1.00	20.60	N
ATOM	644	CA	ILE	103	-1.672	12.580	7.664	1.00	20.83	C
ATOM	645	CB	ILE	103	-0.220	12.115	7.937	1.00	21.14	C

ATOM	646	CG2	ILE	103	0.262	11.190	6.832	1.00	20.31	C
ATOM	647	CG1	ILE	103	0.685	13.350	8.046	1.00	20.27	C
ATOM	648	CD1	ILE	103	2.144	13.056	8.323	1.00	20.70	C
ATOM	649	C	ILE	103	-2.568	11.414	7.248	1.00	20.54	C
ATOM	650	O	ILE	103	-2.897	11.288	6.072	1.00	20.36	O
ATOM	651	N	TYR	104	-2.974	10.569	8.194	1.00	20.06	N
ATOM	652	CA	TYR	104	-3.879	9.475	7.843	1.00	18.57	C
ATOM	653	CB	TYR	104	-4.007	8.411	8.939	1.00	17.25	C
ATOM	654	CG	TYR	104	-2.810	7.512	9.145	1.00	18.10	C
ATOM	655	CD1	TYR	104	-2.836	6.214	8.649	1.00	19.08	C
ATOM	656	CE1	TYR	104	-1.795	5.339	8.849	1.00	18.97	C
ATOM	657	CD2	TYR	104	-1.688	7.920	9.851	1.00	17.72	C
ATOM	658	CE2	TYR	104	-0.621	7.043	10.059	1.00	20.75	C
ATOM	659	CZ	TYR	104	-0.691	5.746	9.551	1.00	20.88	C
ATOM	660	OH	TYR	104	0.323	4.835	9.749	1.00	19.81	O
ATOM	661	C	TYR	104	-5.259	9.907	7.370	1.00	19.95	C
ATOM	662	O	TYR	104	-5.991	9.105	6.794	1.00	21.99	O
ATOM	663	N	ARG	105	-5.626	11.162	7.602	1.00	17.59	N
ATOM	664	CA	ARG	105	-6.915	11.623	7.121	1.00	17.20	C
ATOM	665	CB	ARG	105	-7.491	12.768	7.959	1.00	16.31	C
ATOM	666	CG	ARG	105	-7.847	12.404	9.383	1.00	17.10	C
ATOM	667	CD	ARG	105	-8.413	13.609	10.125	1.00	16.21	C
ATOM	668	NE	ARG	105	-8.799	13.284	11.495	1.00	16.48	N
ATOM	669	CZ	ARG	105	-9.313	14.159	12.358	1.00	19.13	C
ATOM	670	NH1	ARG	105	-9.495	15.427	12.003	1.00	17.52	N
ATOM	671	NH2	ARG	105	-9.676	13.760	13.572	1.00	19.14	N
ATOM	672	C	ARG	105	-6.798	12.023	5.663	1.00	17.20	C
ATOM	673	O	ARG	105	-7.789	12.336	5.021	1.00	16.63	O
ATOM	674	N	ASN	106	-5.574	11.989	5.147	1.00	19.37	N
ATOM	675	CA	ASN	106	-5.302	12.366	3.766	1.00	20.38	C
ATOM	676	CB	ASN	106	-4.507	13.663	3.704	1.00	20.43	C
ATOM	677	CG	ASN	106	-5.251	14.812	4.339	1.00	20.58	C
ATOM	678	OD1	ASN	106	-6.143	15.390	3.723	1.00	23.31	O
ATOM	679	ND2	ASN	106	-4.885	15.160	5.562	1.00	17.80	N
ATOM	680	C	ASN	106	-4.659	11.281	2.919	1.00	21.95	C
ATOM	681	O	ASN	106	-4.027	11.558	1.896	1.00	20.86	O
ATOM	682	N	LEU	107	-4.823	10.042	3.361	1.00	21.77	N
ATOM	683	CA	LEU	107	-4.264	8.899	2.666	1.00	21.73	C
ATOM	684	CB	LEU	107	-2.737	8.860	2.832	1.00	20.40	C
ATOM	685	CG	LEU	107	-2.118	8.762	4.235	1.00	19.18	C
ATOM	686	CD1	LEU	107	-2.558	7.498	4.968	1.00	18.29	C
ATOM	687	CD2	LEU	107	-0.602	8.811	4.171	1.00	16.48	C
ATOM	688	C	LEU	107	-4.943	7.622	3.150	1.00	24.57	C
ATOM	689	O	LEU	107	-5.476	7.574	4.261	1.00	26.59	O
ATOM	690	N	VAL	108	-4.950	6.604	2.300	1.00	24.85	N
ATOM	691	CA	VAL	108	-5.535	5.314	2.637	1.00	23.27	C
ATOM	692	CB	VAL	108	-6.585	4.884	1.594	1.00	25.05	C
ATOM	693	CG1	VAL	108	-6.727	3.374	1.592	1.00	26.97	C
ATOM	694	CG2	VAL	108	-7.923	5.523	1.917	1.00	23.65	C
ATOM	695	C	VAL	108	-4.435	4.259	2.704	1.00	22.36	C
ATOM	696	O	VAL	108	-3.534	4.240	1.866	1.00	23.25	O
ATOM	697	N	VAL	109	-4.494	3.397	3.714	1.00	21.67	N
ATOM	698	CA	VAL	109	-3.504	2.336	3.865	1.00	20.80	C
ATOM	699	CB	VAL	109	-3.455	1.801	5.316	1.00	19.45	C
ATOM	700	CG1	VAL	109	-2.544	0.583	5.389	1.00	17.84	C
ATOM	701	CG2	VAL	109	-2.941	2.892	6.256	1.00	18.14	C
ATOM	702	C	VAL	109	-3.787	1.173	2.910	1.00	21.74	C
ATOM	703	O	VAL	109	-4.915	0.696	2.811	1.00	22.57	O
ATOM	704	N	VAL	110	-2.754	0.739	2.195	1.00	22.61	N

ATOM	705	CA	VAL	110	-2.871	-0.365	1.249	1.00	23.79	C
ATOM	706	CB	VAL	110	-1.791	-0.279	0.158	1.00	22.99	C
ATOM	707	CG1	VAL	110	-1.997	-1.377	-0.864	1.00	23.58	C
ATOM	708	CG2	VAL	110	-1.828	1.081	-0.495	1.00	24.01	C
ATOM	709	C	VAL	110	-2.722	-1.703	1.961	1.00	24.53	C
ATOM	710	O	VAL	110	-3.639	-2.521	1.944	1.00	28.90	O
ATOM	711	OH2	WAT	901	6.275	31.112	15.565	1.00	15.47	O
ATOM	712	OH2	WAT	902	0.081	30.036	10.221	1.00	17.32	O
ATOM	713	OH2	WAT	903	0.127	15.519	19.408	1.00	7.93	O
ATOM	714	OH2	WAT	904	7.030	2.992	5.951	1.00	1.12	O
ATOM	715	OH2	WAT	905	-8.260	9.003	4.793	1.00	16.56	O
ATOM	716	OH2	WAT	906	-0.287	23.410	17.398	1.00	20.45	O
ATOM	717	OH2	WAT	907	11.907	15.641	5.635	1.00	12.75	O
ATOM	718	OH2	WAT	908	6.231	3.121	-2.803	1.00	30.59	O
ATOM	719	OH2	WAT	909	14.427	27.473	-1.777	1.00	32.06	O
ATOM	720	OH2	WAT	910	1.367	29.323	5.015	1.00	30.79	O
ATOM	721	OH2	WAT	911	-3.588	-4.814	0.678	1.00	24.03	O
ATOM	722	OH2	WAT	912	3.828	6.836	-11.268	1.00	35.90	O
ATOM	723	OH2	WAT	913	8.152	2.609	8.906	1.00	34.73	O
ATOM	724	OH2	WAT	914	2.691	4.733	10.966	1.00	33.58	O
ATOM	725	OH2	WAT	916	-5.458	26.444	7.758	1.00	45.56	O
ATOM	726	OH2	WAT	917	-8.076	13.274	16.506	1.00	48.77	O
ATOM	727	OH2	WAT	918	-7.491	17.267	10.217	1.00	40.88	O
ATOM	728	OH2	WAT	919	-3.101	7.362	-4.725	1.00	53.52	O
ATOM	729	OH2	WAT	920	1.887	19.751	-8.003	1.00	32.67	O
ATOM	730	OH2	WAT	921	-8.379	15.505	-1.840	1.00	19.94	O
ATOM	731	OH2	WAT	922	12.181	19.059	-4.016	1.00	25.92	O
ATOM	732	OH2	WAT	923	-8.305	16.544	7.450	1.00	29.31	O
ATOM	733	OH2	WAT	924	-10.379	14.823	6.843	1.00	19.67	O
ATOM	734	C1	SCH	999	14.935	15.343	10.195	1.00	58.18	C
ATOM	735	C2	SCH	999	13.897	15.023	11.252	1.00	58.15	C
ATOM	736	O1	SCH	999	14.212	14.616	12.373	1.00	58.19	O
ATOM	737	N1	SCH	999	12.629	15.230	10.832	1.00	57.26	N
ATOM	738	C3	SCH	999	11.571	15.353	11.839	1.00	54.45	C
ATOM	739	C4	SCH	999	10.855	14.032	12.129	1.00	55.03	C
ATOM	740	O2	SCH	999	11.268	12.976	11.642	1.00	55.46	O
ATOM	741	N2	SCH	999	9.780	14.154	12.934	1.00	56.28	N
ATOM	742	C5	SCH	999	9.102	12.951	13.392	1.00	58.10	C
ATOM	743	C6	SCH	999	10.025	12.199	14.360	1.00	59.43	C
ATOM	744	O3	SCH	999	10.530	11.115	14.068	1.00	59.32	O
ATOM	745	N3	SCH	999	10.203	12.847	15.527	1.00	61.53	N
ATOM	746	C7	SCH	999	11.400	12.542	16.310	1.00	62.93	C
ATOM	747	C8	SCH	999	11.113	11.660	17.534	1.00	62.40	C
ATOM	748	O4	SCH	999	11.912	11.709	18.416	1.00	60.48	O
ATOM	749	O5	SCH	999	10.616	10.479	17.100	1.00	62.41	O
ATOM	750	C9	SCH	999	10.586	16.476	11.453	1.00	49.76	C
ATOM	751	C10	SCH	999	12.105	13.839	16.718	1.00	64.21	C
ATOM	752	C11	SCH	999	13.629	13.756	16.707	1.00	65.67	C
ATOM	753	C12	SCH	999	14.196	14.646	17.803	1.00	66.24	C
ATOM	754	O6	SCH	999	14.526	15.775	17.594	1.00	66.36	O
ATOM	755	O7	SCH	999	14.290	14.052	19.016	1.00	65.17	O
ATOM	756	C13	SCH	999	9.685	16.072	10.295	1.00	46.19	C
ATOM	757	C14	SCH	999	8.313	16.472	10.068	1.00	43.66	C
ATOM	758	C15	SCH	999	7.872	15.829	8.846	1.00	42.20	C
ATOM	759	N4	SCH	999	8.951	15.079	8.368	1.00	43.85	N
ATOM	760	C16	SCH	999	10.006	15.227	9.219	1.00	44.78	C
ATOM	761	C17	SCH	999	7.411	17.318	10.780	1.00	43.03	C
ATOM	762	C18	SCH	999	6.085	17.525	10.294	1.00	39.51	C
ATOM	763	C19	SCH	999	5.651	16.887	9.095	1.00	39.12	C

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the 5 scope of the appended claims.

It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Various publications are cited herein, the disclosures of which are 10 incorporated by reference in their entireties.